REFORESTATION TECHNOLOGY TO RESTORE LONGLEAF PINE FORESTS

By

DANTE ARTURO RODRIGUEZ-TREJO

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This dissertation is dedicated with all my love to:

My wife Lety

With her love, nice temper, discipline and positive attitude to confront the challenges of life, we could go to the top of mount Everest!

My son Dante, Jr.

I am so proud of you because of your outstanding accomplishments in school, on the little league base-ball field, the swimming pool, for skating so well, for enjoying reading, your friends, and life so much!!!

The memory of my mother, Sra. María Dolores Trejo de Rodríguez

Through her love she always made every possible sacrifice for us, her sons and daughters.

My father, Ing. Maximino Rodríguez Aguilar

A man of extraordinary firm temper, with love always inculcated in us in study and sports.

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Abstract of the Dissertation Presented to the Graduate School
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REFORESTATION TECHNOLOGY TO RESTORE LONGLEAF PINE FORESTS

By

Dante Arturo Rodríguez-Trejo

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Chairman: Dr. Mary L. Duryea
Major Department: School of Forest Resources and Conservation

Producing seedlings with specific traits along with altering the configuration of forest gaps may improve reforestation success of longleaf pine seedlings. Seedlings traits such as shoot, root and primary needle length may be good predictors of survival and vigor at the regeneration site. At the Florida planting site, for example, primary needle length and shoot length were correlated with survival one year later in containerized seedlings, and increased root length of bare root seedlings raised survival. Nitrogen fertilization in the nursery did not impact survival but resulted in earlier bud burst in the spring, a possible advantage especially in competitive environments. At the Georgia planting site, where the survival was low due to the drought of 1998, survival was higher in the forest than in small (0.10 ha) and large (1.6 ha) gaps. In the large gaps, survival of

containerized seedlings was higher at the edges, particularly the SW edge. The shade of adult trees and the nurse effect of shrubs increased the survival. Grass competition reduced survival. During dry years the "exclusionary zone" at the edge of gaps may be re-named the "survival zone." A model using oval-shaped gaps oriented from NW to SE, with an area of 0.25 ha is proposed to maximize the survival and growth of longleaf pine regeneration.

CHAPTER 1
INTRODUCTION

Nine thousand years ago, longleaf pine (*Pinus palustris* Mill.) was located in a single area in northeastern Mexico or southern Texas. At the close of the Pleistocene, climate amelioration allowed longleaf pine to migrate northward and eastward, eventually to occupy most of the southeastern United States (Delcourt and Delcourt 1991, Schmidtling and Hipkins 1998).

Longleaf pine has played a major ecological role in the southeastern USA for the last several thousand years. As the climate shifted toward greater rainfall and lightning frequency, the pronounced flamability of the vegetation of this ecosystem apparently contributed to great reduction of habitats dominated by scrub, rearranging countless plant and animals and creating vegetation patterns more favorable to humans (Landers et al. 1990). The growing human population influenced the expansion of the longleaf pine range. Delcourt and Delcourt (1997) mention continuous human use of fire for the past 5,000 years. The effect of anthropogenic fire in southern forests was to increase the extent of longleaf pine (Hamel and Buckner 1998).

Longleaf pine ecosystems are unique in their richness and biodiversity at many scales. The longleaf pine ecosystem is one of the most species-rich plant communities in the world outside the tropics, due to the high number of understory plants per unit area,

reaching in some localities 140/1,000 m² (Peet and Allard 1993, Platt 1998); over 98% of the more than 1,013 taxa in this ecosystem are not trees (Hermann 1995, Drew et al. 1998). Chapman (1932) considered longleaf pine to be possibly the most extensive natural "fire climax" ecosystem dominated by one tree species in the USA.

This ecosystem has at least 187 rare vascular plant species (Walker 1993), at least 36 mammal and 86 bird species (Engstrom 1993), and at least 34 amphibian and 38 reptilian species (Guyer and Bailey 1993). New species continue to be discovered. It has been estimated that 5,000 arthropod species reside in the xeric longleaf pine habitats alone, including 10% endemics. Over 70% of the flowering plants have entomophilous pollination, so pollination processes may also have significant effects on the community (Folkerts et al. 1993). Wildlife in the longleaf pine ecosystem include key stone species, such as the gopher tortoise, whose burrows host some 360 species, including small mammal, snakes, and arthropods (Witz et al. 1991, Palik et al., unpublished) (Figure 1-1). Alteration of fire regimes, exotic species, and other factors may influence the diversity and abundance of all of these native species including arthropods (Folkerts et al. 1993).

Disturbances at many scales help to maintain structure and function of the longleaf pine ecosystem (hurricanes, wildfire, lightning, and burrows made by gopher tortoises, gophers, and ants). Disturbances create the patchy structure and rich biodiversity, and help to sustain the mineral nutrient recycle (Komarek 1968, Palik et al 1995, Hermann 1993, Gordon et al. 1997, Gilliam and Platt 1999).

Figure 1-1. The rich biodiversity of a longleaf pine ecosystem as illustrated by Elliott © (in Moore 1995).

The diversity of physical micro-environments in the longleaf pine range has fostered development and maintenance of high genetic variation among populations or seed sources. Local variation in soils and topography and annual variation in microclimate have resulted in considerable variation among individual trees within populations in traits affecting survival, growth and disease resistance (Snyder et al. 1977, Schmidtling and White 1990). Evidence suggests that longleaf pine has levels of genetic diversity comparable to other widespread conifers (Wells and Snyder 1976, Hamrick et al. 1993).

Although humans historically may have helped to sustain the longleaf ecosystem with fire, beginning about four centuries ago this ecosystem began to diminish in size from its maximum of about 37 million hectares (Frost 1993). Some of the major events leading to the decline of longleaf pine range from the 17th century to present include land clearing, introduction of feral hogs, construction of railroads, logging, and fire suppression (Croker 1987, Frost 1993).

Presently this ecosystem covers less than three million acres (Landers et al. 1995) (Figure 1-2). Florida ranks first among all the USA states in terms of the number of endangered ecosystems, including longleaf pine (Noss and Peters 1995). Also, today there are many endangered species that belong to this ecosystem, with the red-cockaded woodpecker leading the list.

There are many biological and cultural issues that complicate the potential restoration of longleaf pine. Ironically, it was the crucial fire adaptation represented by the longleaf seedlings' grass stage that results in almost no height growth during some

Figure 1-2. The maximum natural potential range of longleaf pine (adapted from Franklin 1997).

years, that was the main factor that historically discouraged landowners from using it in reforestation programs.

Other factors also contribute to making longleaf a uniquely difficult species to successfully reforest. Seed production is episodic, and the seed is susceptible to damage during mechanical sowing. There is a relatively large minimum root collar diameter for acceptable survival in the field (e.g., about 1 cm for bare root seedlings). It is also very sensitive to container size and to competition both in containers and beds. During lifting and packing, its roots are very susceptible to air exposure. Longleaf requires more care during planting and frequent low survival rates have been reported. Before and after planting, inter and intraspecific competition are problems and brown spot disease during the grass stage is relatively common. This disease may be controlled with fire

(Wahlenberg 1946, Croker and Boyer 1975, Boyer and Peterson 1983, Dennington and Farrar 1983, Boyer 1990).

On the other hand, longleaf is very resistant to fire, pests, and diseases (except for needle brown spot). In addition to its ecological importance, it grows fast after leaving the grass stage, and its wood has excellent quality (Boyer and Peterson 1983, Dennington and Farrar 1983, Boyer 1990).

Presently we are seeing the largest ever efforts to restore the longleaf pine ecosystem. For instance, according to Horton (1995), The Nature Conservancy is making longleaf ecosystem recovery one of its major initiatives, and the Department of Defense is trying to restore the longleaf pine ecosystem on 320,000 acres of Eglin Air Force Base, Florida. In addition, the Florida Division of Forestry's nurseries, as many other nurseries, are producing increasing numbers of longleaf pine seedlings (Longleaf Pine Alliance 1998). Scientific institutions are also focusing more on longleaf pine, including The Longleaf Pine Alliance which is dedicated solely to working on the species. Institutions such as the Tall Timbers Research Station, the Joseph W. Jones Ecological Research Center in Georgia, the Southeast Forest Experiment Station (USDA, FS), Auburn University, and the University of Florida are researching longleaf restoration and reforestation and management. Even city governments, such as of Gainesville, Florida, promote the conservation of this ecosystem in city parks.

Despite the increased attention to longleaf pine, there are still many crucial questions to understand this species and ecosystem better and to learn to restore, manage and conserve it. Those questions involve areas such as genetics, nursery and reforestation

technology, ecophysiology, ecology, and restoration. The present dissertation is an attempt to learn more about reforestation and ecology of longleaf pine restoration.

This study had four objectives. Objective (1) was to investigate the morphology and physiology of longleaf pine nursery seedlings, specifically starches, sugars, foliar nitrogen (N) and foliar phosphorous (P) in seedlings fertilized with N in autumn (Chapter 2). Objective (2) was to investigate the effects of nursery fertilization on seedling performance (survival, morphology and growth) after field planting (Chapter 3). Objective (3) was to assess the performance of the N fertilized seedlings in forest gaps with multiple resources, such as light and nutrients (Chapter 4). Objective (4) was to investigate root growth potential tested for several growing conditions (Chapter 5). Finally, these results are integrated and general conclusions and recommendations are presented (Chapter 6).

CHAPTER 2
EFFECTS OF FALL NITROGEN FERTILIZATION ON MORPHOLOGY AND PHYSIOLOGY OF LONGLEAF PINE SEEDLINGS

Introduction

Restoration of longleaf pine (*Pinus palustris* Mill) ecosystems is one of the main conservation objectives in the southern United States. According to Boyette (1996), by the summer of 1995, 59 forestry, wildlife and conservation organizations planned to manage this species in the future. At that time, 28 agencies had an action plan oriented to restoration and regeneration of longleaf pine ecosystems.

Longleaf pine seedling production in forest nurseries has increased dramatically in recent years. From 1996 to 1998, the annual nursery production increased 4.4%, reaching 65 million seedlings (41.2% bare rooted, 58.8% containerized) (Longleaf Pine Alliance 1998).

Many factors contribute to successful nursery production of seedlings, ranging from the seed source used to lifting and storage practices. Longleaf pine has very specific needs in a nursery. Among the main causes of failure in longleaf plantations, low quality seedlings and the lack of competitive vegetation control predominate (Barnett and Mc Gilvray 1997).

The most critical factors to produce longleaf pine seedlings in containers are sowing method, growing medium moisture, and the volume of containers (Brissette et al.

1990). Being intolerant to crowding, longleaf grow bigger in large containers (Barnett and Brissette 1986). The most optimal growth is produced by sowing the seeds in the nursery during the spring, growing seedlings during the summer, hardening them off in the fall, and outplanting in late fall or early winter (van Linde 1990, Brisette et al. 1991, Goodwing 1979).

For bare-root production, the cultural practices to produce larger, more vigorous seedlings, with higher survival rates include: sowing in the fall at a density of 86 to 161 (108 to 129 is ideal)/m^2, mulching to prevent sand splash, vertical pruning of lateral roots by summer and early fall, *Pisolithus tinctorius* (mycorrhizae) inoculation, treating the roots with Benomyl®, fertilizing throughout the growing season, and watering as necessary (Cordell et al. 1990, Goodwing 1979, Gramling 1990, Hatchell and Muse 1990, Kais et al. 1985, Marx et al. 1984, Wilson 1990).

Among the southern pines, longleaf seedlings are the most susceptible to improper handling. For instance, exposure of roots during lifting should be minimized, packing should be carefully done, and the seedlings stored for only short periods (Barnett and Pesacreta 1990, Barnett and McGilvray 1997, Gramling 1990, van Linde 1990, White 1979, Wilson 1990).

Despite the stock type, the massive needle development of this species produces shade, so upper needle clipping is common to allow better exposure of lower needles to light, and to reduce humidity and disease (Brisette et al. 1991). While this practice reduced root-collar diameter and root dry weight by 10%, and shoot dry weight by 50%,

in comparison with unclipped seedlings (Barnett 1984a), clipping increased survival by 13% (South 1998).

High quality seedlings are those that exhibit good survival and growth on a particular site (Duryea 1985). High quality involves interrelated morphological (shape and structure) and physiological (functions and vital processes) traits (Johnson and Cline 1991). In the case of longleaf pine, size has been related to high quality. Several studies reveal a direct correlation between survival and growth size of the seedlings during the first year(s) after planting (e.g., Brown 1964, Kais 1980, White 1981).

Characteristics of containerized longleaf pine seedlings related with good field performance include: a caliper larger than or equal to 0.44 cm, abundant secondary needles, root weight, and shoot weight (Barnett 1984b). In the case of bare-root stock, good characteristics are: root collar diameter larger or equal than 1 cm, at least six primary lateral roots larger or equal than 2 mm in diameter, a fibrous root system, 25% or more of total feeder root ectomycorrhiza, a tap root larger or equal than 15 cm length, and a well-developed white bud (Wakeley 1948, Cordell et al. 1990, White 1979, May 1985).

Information on the nutritional demands of containerized longleaf pine is scarce (Brisette et al. 1990), yet in the nurseries nitrogen (N) is commonly applied to increase seedling size (Fisher and Mexal 1984). However, there are few studies correlating nutritional status to field performance (Mattson 1997), and results so far are contradictory (Duryea and McClain 1984), due to the lack of uniformity and control of other nursery environmental (Lavender 1984) and planting site factors.

Effects of fertilization on longleaf pine are contradictory. For example, fall fertilization increased seedling dry weight, shoot:root ratio, and nutrient concentrations in one year old seedlings, without any effect on survival (Hinesley and Maki 1980). In another study, longleaf pine receiving high N applications in the fall experienced reduced first year survival (Schomaker 1969).

In containerized longleaf pine seedlings, positive correlations were found among stem diameter and the concentration of reducing sugars, shoot dry weight and the concentration of total and reducing sugars, and root dry weight and concentration of total and reducing sugars. Total sugars ranging from 2.5 to 4.6% are associated with acceptable field survival (Barnett 1984b). However, attempts to correlate root starch, sugar and total non structural carbohydrates with field performance have had little success (Duryea and McClain 1984, Ritchie 1984, Johnson and Cline 1991).

Despite important advances in the understanding of how to produce longleaf pine in forest nurseries, and how to obtain better survival, there continue to be establishment failures, particularly in harsh environments or during droughty years. There are still many questions to answer regarding nursery technology, artificial and natural regeneration, and restoration ecology of longleaf pine. This research attempts to help elucidate some questions on longleaf pine nursery production and seedling performance by determining the effects of nursery N fertilization in the autumn on the morphology and physiology (N, phosphorous (P), and carbohydrates) of containerized and bare-root longleaf pine seedlings at the time of planting.

Methodology

Production of Seedlings

Seed for the production of bare-root (BR) and containerized (C) seedlings was collected from International Paper Co. longleaf pine forests near Bainbridge, Georgia (seed lot ICHLL93). The bare-root experiment was installed in October 1996 at a forest nursery in northern Florida. The soils in the nursery correspond to the Hogue series, Hogue fine sand type. These soil series are nearly level, sandy, well drained and deep, with a thin dark colored sandy surface layer over a strong brown and a yellowish red loamy subsoil layer. The sandy clay loam is between 150 and 310 cm. The pH ranges from 4.5 to 6.0 for depths from 0 to 185 cm (USDA Soil Conservation Service 1986).

Seeds for bare-root production were mechanically sown in and covered with mulch. Seedling density was 100 per square meter. The seedlings were irrigated, fertilized, clipped and root-pruned as scheduled for longleaf pine production. The seedlings were lifted mechanically on February 2, 1998 and packaged in bundles of 100 seedling, with hydrated gel applied to maintain root moisture. After storage at 2°C in the Florida nursery for two weeks, the seedlings were transported to the planting site in Georgia.

Two types of containerized seedlings were produced at different nurseries in Georgia and Florida. In Georgia, the seedlings were produced in high density black polyethylene, 40-cavity seedling trays. Each cavity was 4 cm diameter with a volume of 107 cm^3/cavity, and a density of 571/m^2. The growing medium was a mix of peat moss, vermiculite and perlite. Seed was sown in the spring 1997. Irrigation and fertilization

were practiced as routinely scheduled in the nursery. Needles were not clipped. The seedlings were transported to the planting site in February 1998.

The Florida containerized seedlings were produced in the same nursery where the bare root seedlings were produced. The containers were white polyethylene, with 96 cavities per tray, a diameter of 3.9 cm, a volume of 117 cm^3, and a density of 450/m^2. The growing medium was a mix of peat moss, vermiculite and perlite. In spring at the time of sowing, 2.7 kg of fertilizer Osmocot® 17-6-10 (9 months capacity release) per cubic meter of growing media was applied. Nine hundred grams of pelletized soil acidifier (sulfur), was also added. During growth, the seedlings were irrigated as needed, and fertilized as routinely scheduled in the nursery. No clipping of needles was practiced.

Experimental Designs

In the Florida nursery, a randomized complete block experimental design along five contiguous beds was established with five blocks and three N treatments (Figure 2-1). Each block had three treatment plots 5 m long and 1.2 m wide each, with a 0.6 m long buffer zone between them. The treatments consisted of randomly assigned 0, 2, or 4 (no N, low N and high N) biweekly applications of ammonium nitrate (NH_4NO_3), granular and prilled (with 34% of N), applied mechanically in the fall. Each application was 168 kg/ha or 57 kg of N/ha. Six hundred seedlings per treatment and block, 1,800 per block and 9,000 seedlings in total were produced.

The experimental design for the containerized seedlings in Georgia was also a randomized complete block design, with six blocks, each one with three fall N fertilization levels (figure 2-2). One hundred parts per million of N as ammonium nitrate was applied

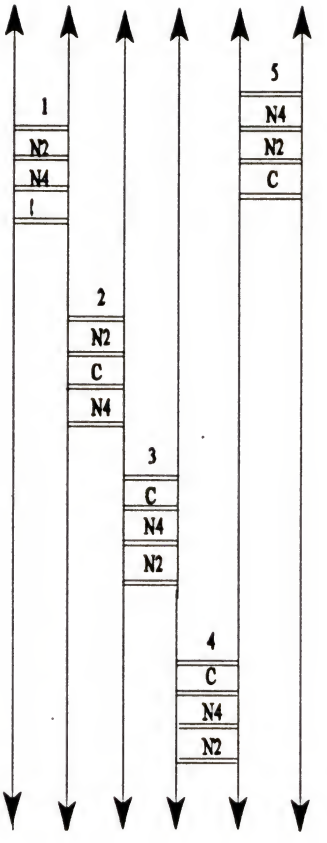


Figure 2-1. At the bare-root nursery, five blocks were randomly assigned to the nursery beds, and the three fertilizer treatments (C=control, N2=two N applications, N4=four N applications) were randomly assigned to each block.

every other week, 0, 2, and 4 times. The solution was applied with a watering can until saturation of the growing media was reached. There were 12 trays per treatment, 36 per block and 216 for the experiment, for a total of approximately 8,640 seedlings.

The experimental design for the containerized seedlings in Florida was a randomized complete block design, with six blocks, each one with three fall N fertilization

a)

4	4	0	2	4	2
0	2	2	4	0	4
2	0	4	0	2	0
1	2	3	4	5	6

b)

0	4	0	2	4	0
2	0	4	0	2	4
4	2	2	4	0	2
4	5	1	3	2	6

Figure 2-2. a) At the Georgia container nursery, the three nursery fertilization treatments were randomly assigned in each of the six blocks; b) At the Florida container nursery, the three nursery fertilization treatments were randomly assigned in each of the six blocks. There were buffer trays surrounding the experimental plots in both cases. The numbers into plots represent the number of fertilizer applications.

levels (Figure 2-2). The application of N treatments was the same as described for the Georgia containers. There were four trays per treatment, 12 per block and 72 for the experiment, for a total of approximately 6,900 seedlings.

Statistical Models

Several statistical models were used to analyze differences in morphology, nutrients and carbohydrates. For morphology and nutrient analysis by stock type the following model was used:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk},$$

where α_i =effect of the i-th N treatment, and β_j =effect of the j-th block. For the carbohydrate analysis by seedling type the following model was used:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \varepsilon_{ijkl},$$

where α_i = effect of i-th N treatment, β_j = effect of the j-th part of the seedling treatment, and γ_k = effect of k-th block.

Regarding the comparison among seedling types, every stock type experiment was developed separately from each other:

$$y_{ijkl} = \mu + \alpha_i + \alpha_i(\beta_j) + \gamma_k + (\alpha\gamma)_{ik} + \alpha_i(\beta_j)\gamma_k + \varepsilon_{ijkl},$$

where α_i = effect of i-th seedling type treatment, $\alpha_i(\beta)_j$ = effect of the j-th block nested within the i-th level of seedling type, γ_k = effect of the k-th N treatment.

The comparison among seedling types, carbohydrates analysis, also involved a nested model:

$$y_{ijklm} = \mu + \alpha_i + \alpha_i(\beta_j) + \gamma_k + \delta_l + (\alpha\gamma)_{ik} + (\alpha\delta)_{il} + \alpha_i(\beta_j)\gamma_k + \alpha_i(\beta_j)\delta_l + (\gamma\delta)_{kl} + \alpha_i\gamma_k\delta_l + \alpha_i(\beta_j)\gamma_k\delta_l + \varepsilon_{ijklm},$$

where: α_i = effect of the i-th stock type, $\alpha_i(\beta_j)$ = effect of the j-th block, nested within the

i-th stock type, γ_k = effect of the k-th N treatment, and δ_l = effect of the l-th part of seedling treatment.

In all of the models, μ represents the overall mean, ε the random error term $(0, \sigma^2)$, and the combinations of letters represent the interactions among the respective factors. The blocks and their interactions and nested effects all have random effects. All of the other factors and interactions have fixed effects.

To control the group wide type I error rate was applied the Bonferroni inequality, as described by Rice (1989). If $p_i \leq \alpha / (1 + k - i)$, then the p_i-value was table-wide significant. p_i = i-th p-value, α=0.10, k=total number of p-values, and i=successive number.

Data Processing

The statistical computations were performed using SAS (Statistical Analysis System®), v. 6.12 for microcomputers, specifically the mixed procedure (proc mixed). These p-values were used as the most meaningful among the parametric tests performed. The degrees of freedom were determined with the Satterthwaite option (ddfm=satterth) (Littell et al 1999). For all of the variables and coefficients of variation, orthogonal contrasts were applied to determine if there were linear or quadratic significant contrasts among the N treatments.

The values of the orthogonal coefficients were taken from Montgomery (1997). In the case of proc mixed, lsmeans was used to obtain the mean values and the standard errors for morphological variables. Also the proc sort and proc means procedures were used to obtain mean values, and in several cases Duncan's multiple ranges tests were used.

The non-parametric variable (bud burst timing) was analyzed with the frequency procedure (proc freq), applying a Chi-square (χ^2) test.

Morphological Variables

One hundred and twenty bare-root seedlings and 144 of each type of the containerized seedlings (from Georgia and Florida) were measured at time of planting (n=408). After obtaining fresh measurements, every sample was separated into shoots and roots, oven-dried at 70°C for five days, and then weighed. In total, 32 variables were measured or calculated for the bare root seedlings and 28 for the containerized seedlings. The measured or calculated variables are shown in table 2-1.

The shoot:root ratio was calculated with dry weights, the sturdiness coefficient dividing stem diameter by height, and the Dickson quality index (DQI) with the model:

$$DQI = tdw / ((h/d) + (sdw/rdw)),$$

where tdw=total dry weight (g), h=height (cm), d=stem diameter (mm), sdw=shoot dry weight (g), and rdw=root dry weight (g).

Physiological Variables

Procedures for both the extraction and determination of sugars and starches were according to the anthrone method (Johnson 1995).

Sugars

Preparation of samples. Eight seedlings were taken at the time of planting per N treatment, block and stock type, for a total of 264 seedlings corresponding to 120 bare root and 144 containerized seedlings. Each plant was separated into four components: needles, stem and bud, tap and lateral roots. Each seedling was dried at 70°C for five

days, and was ground using a electrical grinder (A. H. Thomas Co.®). Up to 4 g of material from each seedling tissue was placed into plastic or glass 20 ml vials to constitute a sample.

For extraction, a subsample of 100 mg (±10%) was taken, weighed with an analytical balance (Model XA, Fisher Scientific®), recorded to the nearest 0.1 mg, and placed into a 25 ml plastic test tube. Twenty five milliliters of 80% ethyl alcohol (CH_3CH_2OH) was added to each test tube with a pipet, and a set with eight test tubes was placed in 80°C water in a Dubnoff metabolic shaking incubater (GCA Precision Scientific®) for 15 minutes. The test tubes were then placed into an automatic superspeed centrifuge (Model SS-3, SurVall®) at 7,500 rpm at 2°C, for 10 minutes. Each liquid sample (supernatant) with the extracted sugars was poured into a 100 ml volumetric flask. After the full procedure was repeated for a total of three times, the volumetric flask was brought up to a 100 ml volume with 80% ethyl alcohol. Every sample was mixed well and 20 ml of it poured into a plastic 20 ml scintillation vial with a conic inner top (to minimize evaporation loss). The vials were stored at 2°C.

Determinations were made using 15 ml glass test tubes, in sets of 16, 40, or 50 samples, plus three standards and a blank. Every sample had a duplicate. The blank was 5 µl of ethyl alcohol, and the standards included 1 µl, 2.5 µl, and 5 µl of 0.01% glucose standard with 4 µl, 2.5 µl and 0 µl of ethyl alcohol, respectively. The standards were equivalent to 0.01, 0.025, and 0.05 mg of glucose. Five microliters of sample from the scintillation vials was placed in each test tube. Ten milliliters of anthrone reagent was added to each test tube and then they were placed in a boiling water bath for 15 minutes.

The samples were then cooled for two minutes at room temperature and for two minutes in an ice bath. The readings in the spectrophotometer (model 340, Sequoia Turner®, calibrated at 620 nm) immediately followed. Every set was calibrated after warming up the device for 30 minutes, first setting zero for transmittance and then fitting zero by absorbance, using deionized water. Then the readings for blanks and standards to three decimals were obtained.

A linear regression, using the SAS (Statistical Analysis System®) program, version 6.12 was used in each set to check if the R^2 was ≥ 0.95. If so, the samples were read; if not, the set was run again until reaching 0.95 or higher value. The readings from the spectrophotometer were transformed to milligrams of glucose using a linear model for each set, and then calculating back to account for dilution, using the model:

mg glucose/g dry weight = (mg glucose from regression) (200) / g dry weight.

The glucose standard was prepared adding 100 ml of glucose to 100 ml of 95% ethyl alcohol, to make 0.1% solution. To obtain the 0.01% solution 45 ml of 95% ethyl alcohol were added to 5 ml of the 0.1% sugar solution. Both were stored at 2°C. The anthrone reagent was prepared adding 1 g of 98% anthrone ($C_{14}H_{10}O$) powder (Across®) to 1 liter of 70% sulphuric acid (H_2SO_4), mixing into solution, and then storing at 2°C. This reagent was prepared as needed, avoiding use if older than 1.5 days.

Starches

The remnant tissue from the sugar extractions was used to extract starches. A set contained 16 samples, plus a control and a blank. The tissue was stored at 2°C for no more than one day. A control was prepared with 10 mg of soluble starch reagent

$(C_6H_{10}O_5)_n$ (Fisher®). To each sample, and the control and blank, 5 ml of deionized water were added. The set was placed in a boiling water bath for 30 minutes, and then left to cool at room temperature. Then 5 ml of the enzyme amyloglucosidase solution were added to each sample with a repipet, and the samples were left incubating in a shaker bath at 37°C in a water bath (model 25, GCA/Precision Scientific®), for two hours, swirling gently three times during that time period. Once the incubation was completed, the set was placed in a boiling water bath for five minutes to stop the enzyme reaction. The starch hydrolysis produces glucose in this extraction.

The extract was vacuum filtered through a #1 filter paper in volumetric flasks, and then brought to 20 ml with deionized water, rinsing the flask three times during the process. The samples were poured into 20 ml plastic scintillation vials with an inner conic cup, to prevent loss by evaporation. The samples were then stored at 2°C. The amyloglucosidase was prepared adding 1 gram of amyloglucosidase (Glucoamylase, a, 4, α -D-Glucanglucohydrolase, grade II from *Rhizopus*, Sigma®) enzyme powder, per liter of citrate buffer 0.05M and with a pH=4.5. The enzyme was mixed with the citrate buffer for 30 minutes in a mixer. The liquid was vacuum filtered to obtain the clean amyloglucosidase solution. The citrate buffer was prepared adding 0.05M citric acid (5.25 g of granular citric acid $(HOCCOOH(CH_2COOH)_2.H_2O)$, and dissolved in 500 ml of deionized water), to 0.05 M sodium citrate (7.40 g of sodium citrate $(Na_3C_6H_5O_7.2H_2O)$, dissolved in 500 ml of deionized water, until the pH reached 4.5). For measuring the pH a pH-meter (model 240, Corning®), was used. The citrate buffer was stored at 2°C.

Sets (racks) with 32 duplicated samples plus a blank and three standards were prepared for determinations. Five micro liters of deionized water were used for the blank. Four micro liters of deionized water and 1 µl of 0.01% glucose solution were used for the first standard. For the second standard 2.5 µl of deionized water and 2.5 µl of 0.01% glucose solution were used. For the third standard 5 µl of 0.01% glucose standard were used.

With every eight samples a control and a blank for the enzyme, both obtained during the starch extraction process, were included. Except for the blank and the standards, in every 10 ml test tube, 2.5 µl of deionized water and 2.5 µl of every sample were repipeted. Two point five micro liters of deionized water were added to test tubes, each with 2.5 µl of control and 2.5 µl of enzyme blank, both from the extractions. Ten milliliters of anthrone reagent were then added to each test tube. Each set was placed in a boiling water bath for 15 minutes. After that time, the set was left to cool for two minutes at room temperature and then for two more minutes in an ice bath. The next step was to read the blank and the three standards in the spectrophotometer and to run a linear regression, using SAS to verify if the R^2 had a value ≥ 0.95. If not, the process was repeated as described above.

The 0.01% glucose standard was prepared adding 45 ml of deionized water to 5 milliliters of commercial 0.1% glucose solution. To calculate the concentration of starch in mg of starch per gram of dry tissue, the following models were used in this order:

$$sew = (a + b(c))\ 400$$

$$\%ce = (10/sew)100$$

$ge = (a + b(d))\ 400$

$s = (ge/dw)\ (cw/\%ce)$,

where: sew is starch equivalent weight; a, and b are the intersection to the Y-axis and the slope, both from the linear regression in the set, c is the average absorbance for the control starch sample; %ce is the percentage of conversion efficiency; ge is glucose equivalent per sample (mg), d is the average absorbance for the sample; s is the milligrams of starch per gram of dry weight of tissue per sample, dw is the dry weight of the sample, in grams, and cw is the dry weight of the control starch, in milligrams.

Nitrogen

Tissue N content was determined according to the micro Kjeldhal method (Bremner and Mulvaney 1982). For the digestion, every set contained 36 to 40 samples with three standards and a blank. Pine needles were used as standards (Standard reference material NBS 1575), with 1.2% of N, and 0.12% P. Fifty milligrams ± 1 mg of needle tissue were placed in each 50 ml tube test, except in the blank. The tissue was weighed with an analytical balance (Fisher Scientific®), and recorded. Two milliliters of 95% sulphuric acid (H_2SO_4) were placed into each sample using a repipet dispenser. Then, each sample was placed in a heater unit (BD40, Technicon Instrument Co.®), with forty cavities for 30 minutes at 380°C. The samples were removed and left to cool at room temperature for 15 minutes. Then four drops of hydrogen peroxide (H_2O_2), 30% aqueous solution, were added as catalyst to hasten the reaction, and the tubes were placed again in the heater unit, for ten minutes. This last step was repeated three times. After cooling, nutrient determinations were made.

Determination. Ammonium was recovered by distillation. The digest was diluted with distilled water and cooled. Alkali was added, then sodium hydroxide (NaOH) was added in a flask fitted with an efficient trap, and connected to a condenser. Four percent boric acid (H_3BO_3) was used for the absorption of the ammonia from the distillation. The boric acid solution changes from bluish purple to bluish green with the introduction of ammonia. The solution was titrated with 0.1N hydrochloric acid (HCl) until the solution turned from bluish to pink. All the titration was recorded, then the following model was used to determine the percent of N in the sample (%N):

$$\%N = ((bt) (san) (1.4)) / dw = ((t-0.0533) (0.0069) (1.4)) / dw$$

$$bt = t - 0.0533,$$

where bt is the blank titration (ml), san is the sulphuric acid normality, dw the dry weight (g) of sample, and t is the titration of the sample (ml).

Phosphorous

The digestion was conducted in the same way as for the N, using the same standard reference material. The Murphy-Raleigh method was used for the determination. The sulphuric acid reagent A was prepared, adding 179 ml of sulphuric acid, 15 g of ammonium molybdate $(NH_4)_6Mo_7O_{24}$, and 0.364 g of antimony potassium tartrate ($C_8H_4K_2O_{12}Sb_2$), making up four liters. Then 250 ml of this reagent was added to 1.32 g of ascorbic acid ($CH_2OHCHOHCHCOH{:}COHCOO$). Next 25 ml of the digested material were added, and 2 ml of this product were placed in a volumetric flask, with two drops of 0.1% p-nitro phenol ($C_6H_5NO_3$). Then 8 N sodium hydroxide (NaOH) was added, one drop at a time, until the solution changed to yellow color, to back titrate with 1 M

hydrochloric acid (HCl) until clear. Four milliliters of reagent B (ascorbic acid) were added. Finally, the volume was brought up to 25 ml with deionized water, and the sample was incubated for 30 minutes, to be read in the spectrophotometer, at 880 nm.

Five standards per rack (40 samples) were run, with concentrations of 2, 4, 10, 16, and 20 µg P/25 ml. A linear regression of these values with the spectrophotometer readings was used to estimate the concentration of P in 25 ml of solution. To obtain the percent of P, the following model was used:

$$\%P = ((\mu g\ P) (1.25)) / dw,$$

where µg P is the micrograms of P from the linear model and dw is the dry weight of the sample (mg).

Results

Morphological Variables

Bare root seedlings

Only two seedling variables exhibited statistically significant differences (at $p \leq 0.05$ level) and linear trends among the different levels of fall N application (Table 2-1). Bud diameter was greatest for higher levels of N fertilization, with a linear trend. Shoot length increased with level of N fertilization, following a linear trend.

The high levels of N fertilization resulted in earlier bud break. By the time of lifting, 45% of the bare root seedlings had broken buds after four fall N applications, 47.5% with two applications, and 5% for the control (Chi square, $p=0.0010$).

Table 2-1. Means and p-values for morphology of bare-root seedlings at 0, 2, and 4 fertilizer applications.

Variable	Number of fall fertilizer applications					
	0	2	4	p-value	p-lin	p-quad
Shoot						
Stem diameter (mm)	13.80	14.15	14.36	0.4573	0.2254	0.8485
Stem length (cm)	1.77**b**	1.99**ab**	2.11**a**	0.2550	0.1099	0.7706
Shoot length (cm)	3.02**b**	3.39**a**	3.60**a**	**0.0064**	**0.0018**	0.5802
Bud number	3.40	3.70	3.72	0.7392	0.4857	0.7332
Bud length (mm)	12.47**b**	14.04**ab**	14.88**a**	0.1064	0.0422	0.6859
Bud diameter (mm)	10.29**b**	11.81**a**	12.06**a**	**0.0001**	**0.0001**	0.0698
Needles						
Secondary, number	155.35	180.17	185.07	0.1711	0.0801	0.4957
Secondary, length (cm)	34.47	34.52	35.97	0.4916	0.3044	0.5706
Primary, number	27.75	35.64	26.32	0.4638	0.9479	0.2248
Primary, length (cm)	7.67	7.90	8.82	0.7107	0.4443	0.7658
New, number	0	2.12	0.64	0.2299	0.6149	0.1024
New, length (cm)	0	0.35	0.12	0.4222	0.6432	0.2198
Roots						
Lateral, number	21.65	23.07	22.77	0.4029	0.3148	0.3714
Lateral, length (cm)	30.22	30.65	29.72	0.5120	0.5326	0.3362
Tap length (cm)	18.04**b**	18.88**a**	18.52**ab**	0.1120	0.2382	0.0757
Lateral, pruned (number)	5.12	4.80	4.97	0.9316	0.8659	0.7458
Lateral, pruned length (cm)	26.52	20.12	24.75	0.1688	0.6106	0.0697
Biomass(g)						
Stem	1.62**b**	1.88**ab**	2.00**a**	0.1235	0.0519	0.6314
Secondary needle	8.11	8.50	9.30	0.4293	0.2148	0.8126
Primary needle	0.28	0.35	0.26	0.6831	0.8697	0.3921
New needle	0	0.01	0.01	0.3228	0.1690	0.5453
Shoot (needles+stem+ bud)	10.02	10.76	11.59	0.3805	0.1785	0.9772
Tap	4.72	4.70	4.99	0.6781	0.4691	0.6167

Table 2-1--Continued.

Variable	Number of fall fertilizer applications					
	0	2	4	p-value	p-lin	p-quad
Lateral root	3.30	3.10	3.45	0.8209	0.7942	0.5803
Pruned root	0.46	0.37	0.52	0.2848	0.5153	0.1487
Root (tap+laterals)	8.03	7.92	8.44	0.8415	0.6558	0.7137
Tap dry weight + lateral + pruned roots	8.49	8.33	8.97	0.8286	0.6438	0.6993
Dead biomass	0.66	0.64	0.78	0.4237	0.3182	0.4022
Total	18.05	18.69	20.04	0.5798	0.3194	0.8508
Ratios and indices						
S:R	1.29	1.40	1.42	0.4617	0.2569	0.6567
Sturdiness coefficient	0.21**b**	0.24**ab**	0.25**a**	0.1605	0.0714	0.5849
Dickson quality index	14.76	14.15	14.86	0.9178	0.9594	0.6872

0, 2 and 4 correspond to the number of applications of ammonium nitrate at a doses of 168 kg/ha. p-value calculated with proc mixed, p-lin and p-quad are the p-values for linear and quadratic contrasts for N treatments (proc mixed). Bolded numbers have a p-value equal or lower than 0.05, and still were significant after the Bonferroni inequality at a table-wide α=0.10. In each row, the values with different letters exhibited differences according to the Duncan's multiple ranges test, with a $p \leq 0.05$ obtained from proc glm. (In the case of stem length, p=0.0580).

Containerized seedlings

In the seedlings from Georgia, only one variable exhibited differences (Table 2-2). Length of new needles increased linearly with higher levels of N. In seedlings from Florida, only shoot length (stem length + bud length) exhibited differences among N treatments, with the peak values at the low N level, following quadratic trends (Table 2-3).

Table 2-2. Means and p-values for morphology of containerized seedlings (Georgia) at 0, 2, and 4 fertilizer applications.

Variable	Number of fall fertilizer applications 0	2	4	p-value	p-lin	p-quad
Shoot						
Stem diameter (mm)	11.06	11.23	10.80	0.8299	0.7178	0.6365
Stem length (cm)	1.17	1.34	1.25	0.3725	0.5003	0.2206
Shoot length (cm)	2.01**b**	2.28**a**	2.19**ab**	0.0568	0.1070	0.0747
Bud number	3.02	2.52	2.94	0.6018	0.8754	0.3312
Bud length (mm)	8.43	9.44	9.48	0.1841	0.1059	0.3749
Bud diameter (mm)	8.93**b**	9.89**a**	9.19**ab**	0.3474	0.6959	0.1667
Needle						
Secondary, number	33.5	36.4	37.21	0.5259	0.2857	0.7376
Secondary, length (cm)	40.5	41.83	42.15	0.3963	0.2087	0.6433
Primary, number	13.35**b**	22.44**a**	13.88**b**	0.0310	0.8918	0.0086
Primary, length (cm)	10.67	11.90	11.06	0.5734	0.7394	0.3177
New, number	29.19	29.67	27.77	0.9259	0.7812	0.7881
New, length (cm)	6.23**b**	9.00**a**	10.27**a**	**0.0021**	**0.0007**	0.3249
Roots						
Lateral, number	24.23**a**	21.88**b**	21.33**b**	0.1269	0.0582	0.4702
Lateral, length (cm)	22.52	22.27	22.50	0.9788	0.9878	0.8393
Tap length (cm)	7.84	7.81	7.93	0.8690	0.6824	0.7465
Biomass(g)						
Stem	0.48	0.53	0.50	0.7826	0.7523	0.5425
Secondary needle	2.01	2.30	2.24	0.1785	0.1659	0.2153
Primary needle	0.12**b**	0.24**a**	0.14**b**	0.0043	0.5404	0.0012
New needle	0.23**b**	0.38**a**	0.34**a**	0.0245	0.0365	0.0463
Shoot	2.84**b**	3.45**a**	3.22**ab**	0.0550	0.1091	0.0538
Tap	1.33**ab**	1.53**a**	1.18**b**	0.1389	0.3908	0.0722
Lateral root	1.13	1.23	1.16	0.5562	0.7891	0.2947
Root	2.45**a**	2.76**b**	2.34**a**	0.1041	0.5610	0.0409
Dead biomass	0.40**a**	0.38**ab**	0.32**b**	0.2392	0.1056	0.7122
Total	5.29**b**	6.21**a**	5.56**ab**	0.0419	0.4697	0.0159

Table 2-2--Continued.

Variable	Number of fall fertilizer applications 0	2	4	p-value	p-lin	p-quad
Ratios and indices						
S:R ratio	1.29**b**	1.35**ab**	1.58**a**	0.1436	0.0679	0.4783
Sturdiness coefficient	0.19	0.21	0.21	0.4708	0.3113	0.4958
Dickson quality index	4.68**a**	5.18**b**	4.20**a**	0.1279	0.3163	0.0775

See foot of table 2-1 for notes. In this case the dose was 100 ppm of N, applied as ammonium nitrate solution.

Comparison of bare-root and the containerized seedlings

Twenty-five variables exhibited significant differences among seedling types (Table 2-4). The variables that did not show differences were stem length, shoot length, and sturdiness coefficient. In the case of N treatments, only one variable had significant differences: new needle length, having larger values at higher N doses.

Nitrogen

Bare-root seedlings

Foliar N concentration increased with increasing levels of fertilization (0.81%, 0.97%, and 1.11%, for levels 0, 2, and 4) (p=0.0011) with a significant linear trend (p=0.0003). The coefficients of variation of foliar N also exhibited differences among fertilization levels (p=0.0394), with a quadratic trend (p=0.0444) (15.90%, 18.45%, and 10.76%, for levels no N, low N and high N).

Table 2-3. Means and p-values for morphology of containerized seedlings (Florida) at 0, 2, and 4 fertilizer applications.

Variable	Number of fall fertilizer applications					
	0	2	4	p-value	p-lin	p-quad
Shoot						
Stem diameter (mm)	10.10	9.86	10.09	0.9703	0.9911	0.8093
Stem length (cm)	1.03**b**	5.21**a**	1.00**b**	**0.0280**	0.9867	**0.0085**
Shoot length (cm)	8.22**b**	13.11**a**	8.84**b**	**0.0001**	0.4140	**0.0001**
Bud number	1.69	1.38	1.83	0.1976	0.5376	0.0874
Bud length (mm)	7.19	7.90	7.83	0.7482	0.5377	0.6662
Bud diameter (mm)	8.43	8.48	8.75	0.9537	0.7795	0.9080
Needle						
Secondary, number	40.17	34.56	33.67	0.5020	0.2851	0.6496
Secondary, length (cm)	34.38	34.42	34.52	0.9983	0.9557	0.9890
Primary, number	12.02	8.19	11.54	0.4795	0.8885	0.2336
Primary, length (cm)	8.92	9.71	9.71	0.8965	0.6902	0.8177
New, number	24.54**b**	28.17**ab**	31.85**a**	0.4403	0.2082	0.9621
New, length (cm)	2.42**b**	3.71**a**	4.21**a**	0.1595	0.0685	0.6139
Roots						
Lateral, number	32.39	33.98	31.88	0.6585	0.8647	0.3796
Tap, length (cm)	11.34	11.21	11.20	0.5945	0.3702	0.6548
Biomass (g)						
Stem	0.48**a**	0.38**b**	0.43**ab**	0.5801	0.5780	0.3837
Secondary needle	1.92**a**	1.55**b**	1.66**ab**	0.4750	0.3982	0.3287
Primary needle	0.13**a**	0.10**b**	0.18**ab**	0.3398	0.3573	0.2519
New needle	0.15**b**	0.19**b**	0.26**a**	0.2004	0.0805	0.7897
Shoot	2.69**a**	2.22**b**	2.54**ab**	0.5553	0.7128	0.3147
Tap	1.76	1.53	1.58	0.6277	0.4681	0.5328
Lateral roots	1.21	1.24	1.26	0.9370	0.7234	0.9795
Roots	2.97	2.77	2.83	0.8404	0.6985	0.6644
Dead biomass	0.22	0.23	0.19	0.7714	0.6857	0.5587
Total	5.65	4.99	5.41	0.6698	0.7190	0.4199

Table 2-3--Continued.

Variable	Number of fall fertilizer applications					
	0	2	4	p-value	p-lin	p-quad
Ratios and indices						
S:R ratio	0.95	0.84	0.90	0.4824	0.5969	0.2837
Sturdiness coefficient	1.02**b**	1.37**a**	0.89**b**	0.1840	0.6287	0.0789
Dicskon quality indice	5.90	5.53	5.68	0.8514	0.7335	0.6582

See foot of table 2-1 for notes. In this case, the dose was 100 ppm of N applied as ammonium nitrate solution.

Containerized seedlings

The foliar N concentration was higher when N was added (0.69%, 0.88%, 0.87% for the treatments no N, low N and high N) (p=0.0095), following a linear trend (p=0.0088). The coefficients of variation did not show differences among the N fertilization levels (p=0.2841).

Comparison among containerized and bare root seedlings

There were differences in foliar N among seedling types (p=0.0071), with 0.96% N for the bare root seedlings and 0.81% N for the containerized seedlings, and also there were differences for N fertilization (p=0.0001), with means equal to 0.74%, 0.92%, and 0.98% for levels no N, low N and high N, respectively. There was no significant seedling type by N fertilization interaction (p=0.1173). There was a difference among the seedling types for the coefficient of variation of foliar N (p=0.0331), however. The variation was larger for the containerized seedlings (25.42%), than for the bare root seedlings (20.58%).

Phosphorous and P:N Relationships

In the analysis by seedling type, larger P concentrations for bare root seedlings were found at higher N levels (0.097% P, 0.111% P, 0.115% P, for no N, low N and high N) (p=0.0026), with a linear trend (p=0.0026). In the containerized seedlings, no differences in P concentrations among fertilization treatments were found (0.305% P, 0.284% P, 0.267% P, for no N, low N and high N) (p=0.1352). In the pooled analysis, such trends manifested as seedling type by N treatment interaction (p=0.0170). The P concentration for bare-root seedlings was 0.108%, and 0.285% for containerized seedlings.

In the bare-root seedlings, the P:N ratio exhibited differences (p=0.0310), with values of 0.127, 0.117, and 0.105 for levels no N, low N and high N, and a linear trend (p=0.0096). There was a positive correlation among N and P concentrations (r=0.5072, p=0.0001). In the containerized seedlings, higher N levels corresponded to lower P:N ratios, with 0.451, 0.350, and 0.329 for no N, low N and high N (p=0.0163, linear contrast with p=0.0075). There was no correlation among N and P concentrations (r= - 0.14721, p=0.0783). For the pooled analysis the interaction of seedling type and N treatment (p=0.0262) was significant. Overall, a low but significant negative correlation among N and P concentrations was detected (r= -0.2940, p=0.0001). The mean P:N value for bare root seedlings was 0.116, and 0.377 for containerized seedlings.

Table 2-4. Comparisons among the different types of seedlings.

Variable	Seedling type				Number of fertilizer applications						
	BR	C(GA)	C(FL)	p-see	0	2	4	p-nit	p-lin	p-quad	p-int
Stem											
Diameter (mm)	14.10**a**	11.03**b**	10.01**c**	**0.0001**	11.66	11.75	11.77	0.9698	0.8196	0.9264	0.9317
Stem length (cm)	1.96**a**	1.25**b**	2.41**a**	0.1228	1.32**b**	2.85**a**	1.45**b**	0.0797	0.8201	0.0054	0.0086
Shoot length (cm)	3.34**a**	2.16**b**	3.18**a**	0.0931	2.26**b**	3.89**a**	2.53**b**	0.0151	0.6403	0.0044	0.0085
Bud number	3.60**a**	2.82**b**	1.63**c**	**0.0008**	2.70	2.53	2.83	0.4329	0.5857	0.2401	0.6955
Bud length (mm)	13.80**a**	9.11**b**	7.64**c**	**0.0001**	9.37**b**	10.46**a**	10.75**a**	0.0253	0.0103	0.3744	0.6998
Bud diameter (mm)	11.39**a**	9.33**b**	8.55**c**	**0.0001**	9.22**b**	10.06**a**	10.01**a**	0.1563	0.1069	0.2868	0.5571
Needle											
Secondary, number	173.53**a**	35.70**b**	36.13**b**	**0.0001**	76.35	83.70	85.02	0.2137	0.1036	0.5122	0.0696
Secondary, length (cm)	34.91**b**	41.50**a**	34.44**b**	**0.0005**	36.47	36.93	37.46	0.6577	0.3646	0.9709	0.9660
Primary, number	29.34**a**	16.56**b**	10.58**c**	**0.0001**	17.04	22.09	17.35	0.1981	0.9200	0.0741	0.2797
Primary, length (cm)	8.13**b**	11.21**a**	9.44**b**	**0.0059**	9.09	9.83	9.87	0.6188	0.3899	0.6458	0.9590
New, number	0.93**b**	28.88**a**	28.13**a**	**0.0001**	17.91	19.92	20.10	0.6667	0.4207	0.6949	0.7079
New, length (mm)	0.15**a**	8.50**b**	3.44**c**	**0.0001**	2.88**b**	4.35**a**	4.87**a**	**0.0003**	**0.0001**	0.2199	0.0235
Roots											
Lateral, number	22.49**b**	22.48**b**	32.72**a**	**0.0001**	26.06	26.31	25.32	0.6185	0.4835	0.4951	0.3928
Lateral, length (cm)	30.26**a**	22.43**b**		**0.0001**	26.37	26.46	26.20	0.9474	0.8305	0.8054	0.8276
Tap length (cm)	18.47**a**	7.86**b**	11.25**c**	**0.0001**	12.41	12.63	12.54	0.3298	0.3881	0.2256	0.1167
Lateral pruned, number	4.97										
Lateral pruned, length (cm)	23.80										

Table 2-4--Continued.

Variable	Seedling type				Number of fertilizer applications						
	BR	C(GA)	C(FL)	p-see	0	2	4	p-nit	p-lin	p-quad	p-int
Biomass (g)											
Stem	1.84**a**	0.50**b**	0.43**b**	**0.0001**	0.86	0.93	0.98	0.2404	0.0968	0.8314	0.0943
Secondary needle	8.64**a**	2.18**b**	1.71**c**	**0.0001**	4.02	4.12	4.39	0.4147	0.2014	0.7482	0.3466
Primary needle	0.30**a**	0.17**b**	0.14**b**	**0.0002**	0.17	0.23	0.20	0.3566	0.6002	0.1832	0.2663
New needle	0.01**a**	0.32**b**	0.20**c**	**0.0001**	0.13**b**	0.19**a**	0.20**a**	0.0128	0.0064	0.2348	0.1757
Shoot(needles+stem+bud)	10.79**a**	3.16**b**	2.48**c**	**0.0001**	5.18	5.48	5.77	0.2795	0.1136	0.9842	0.3289
Tap	4.82**a**	1.35**b**	1.62**c**	**0.0001**	2.60	2.59	2.59	0.9942	0.9449	0.9351	0.3458
Lateral root	3.30**a**	1.17**b**	1.23**b**	**0.0001**	1.88	1.86	1.96	0.8104	0.6277	0.6715	0.8662
Pruned root	0.45										
Roots (tap+lateral)	8.16**a**	2.52**b**	2.86**b**	**0.0001**	4.49	4.49	4.56	0.9654	0.8092	0.9150	0.7167
Roots (pruned) (tap+lateral+pruned)	8.64**a**	2.52**b**	2.86**b**	**0.0001**	4.64	4.64	4.73	0.9449	0.7695	0.8708	0.7161
Dead biomass	0.69**a**	0.37**b**	0.21**c**	**0.0001**	0.43	0.42	0.43	0.9533	0.9665	0.7613	0.3851
Total	18.94**a**	5.69**b**	5.34**b**	**0.0001**	9.67	9.98	10.34	0.5663	0.2909	0.9652	0.4898
Ratios and indices											
S:R ratio	1.37**a**	1.41**a**	0.90**b**	**0.0001**	1.18**b**	1.19**ab**	1.30**a**	0.1986	0.1002	0.4668	0.2696
Sturdiness coefficient	0.24**b**	0.21**b**	0.35**a**	0.0775	0.21**b**	0.37**a**	0.22**b**	0.0289	0.8987	0.0082	0.0141
Dickson quality index	14.64**a**	4.70**b**	6.19**c**	**0.0001**	8.62	8.47	8.45	0.9557	0.7849	0.9032	0.8212

BR=bare-root seedlings, C(GA)=containerized seedlings from Georgia, C(FL)=containerized seedlings from Florida, p-see = the p-value (from proc mixed) for seedling types, 0, 2, and 4 = the number of fall N applications (168 kg of ammonium nitrate for bare root seedlings, 100 pm of N as ammonium nitrate in solution for the containerized seedlings), p-nit = p-value for N treatments (proc mixed), p-lin and p-quad = p-values for linear and quadratic contrasts among N treatments, and p-int = p-value for the interaction among seedling type and N treatment. Bolded values are significant after the Bonferroni test.

Sugars

Bare-root seedlings

Nitrogen treatment showed interactive, but not main effects on seedling tissue N (Table 2-5). When seedling part was individually analyzed, only the stems exhibited differences in sugar concentration among N fertilization levels, with a linear trend ($p=0.0001$) (Table 2-6).

Containerized seedlings

There were no differences in sugars among N fertilization levels, but there were differences among the parts of the seedlings. The N treatment by seedling part interaction was not significant (Table 2-7). The coefficient of variation for the sugar concentration exhibited differences among seedlings ($p=0.0017$).

Comparison of containerized and bare-root seedlings

The interaction of seedling type by part was significant ($p=0.0001$) for sugar concentration. No differences were found among fertilization treatments ($p=0.3734$), seedling type by N treatment interaction ($p=0.6825$), fertilization treatment by part of the seedling interaction (0.5639), nor for the interaction of seedling type by N treatment by seedling part ($p=0.1320$) (Table 2-6).

Starch

Bare-root seedlings

There were no differences in starch concentrations among N fertilization levels ($p=0.2099$), but there were differences in starch concentrations among the different parts of the seedlings, as shown in figure 2-3 ($p=0.0001$). The largest concentrations were

Table 2-5. Sugar concentration (mg/g dry weight) by N fertilization treatment and seedling part for the bare-root seedlings.

N treatment:	0	2	4	p-value
	117.12**a**	106.22**b**	113.34**a**	0.5355

Part:	lateral roots	needles	stem	tap root	p-value
	136.95**a**	43.64**b**	104.57**c**	163.76**d**	**0.0001**

N treatment by part interaction	**0.0244**

p-values from proc mixed. 0, 2, 4 = number of fertilizer applications (168 kg/ha of ammonium nitrate). The values with different letters were different (p=0.05) according to the Duncan's multiple range test (proc glm).

Table 2-6. Sugar concentration (mg of sugar/g of dry weight) for the parts of the seedlings and N treatments.

Part	Containerized seedlings N treatment			p-value	Bare root seedlings N treatment			p-value
	0	2	4		0	2	4	
Lateral roots	61.4	56.6	54.5	0.2949	143.2**a**	129.6**b**	138.1**ab**	0.5335
Needles	55.6	52.6	53.4	0.7504	45.3	42.4	43.1	0.7437
Stem	117.3	110.0	115.0	0.5681	111.8**a**	103.0**ab**	98.9**b**	**0.0001**
Tap	138.5	140.1	136.4	0.8739	168.2**a**	149.9**b**	173.3**a**	0.1067

p-values, from proc mixed. Duncan's multiple ranges test per seedling type (proc glm).

found in the tap root and lateral roots, followed by stems and needles. Clearly, roots have more starch than shoots by the end of fall. Analyzing every seedling part separately, there was a significant effect of N treatments on starch concentrations in lateral roots (p=0.0124), with a linear trend (p=0.0062) and high starch concentrations corresponding to low N levels. Neither needles (p=0.9047) nor stems (p=0.6407) exhibited differences

Table 2-7. Sugar concentration (mg/g dry weight) by N fertilization treatment and part of the seedling for the containerized seedlings (from Georgia).

N treatment:	0	2	4	p-value
	92.94	89.79	89.80	0.6666
Part:	**lateral roots**	**needles**	**stem**	**tap root**	**p-value**
	57.47**c**	53.83**c**	114.08**b**	137.99**a**	**0.0001**
N treatment by part interaction					0.9305

p-values from proc mixed. 0, 2, 4 = number of fertilizer applications (100 ppm of N, applied as ammonium nitrate solution). The values with different letters exhibited significant differences (p=0.05) according to the Duncan's multiple range test (proc glm).

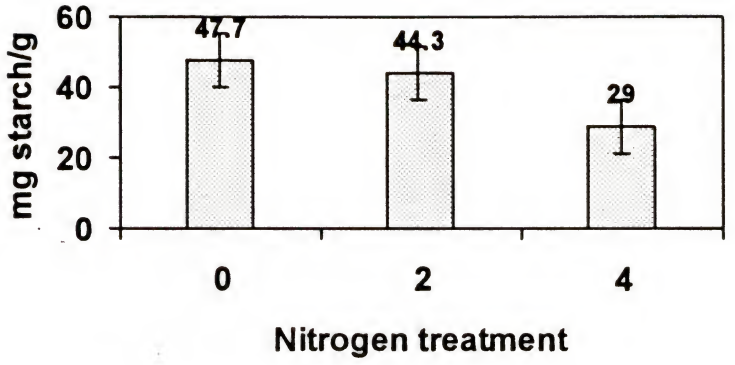
in starch concentration among N treatments. The tap-root showed no differences (p=0.1253), but it had a linear contrast with a p=0.0594 (Figure 2-3).

Containerized seedlings

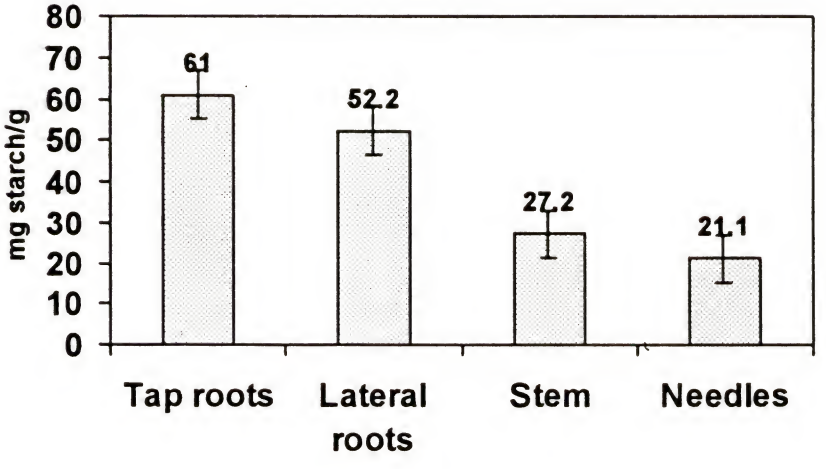
Starch concentrations were significantly different among N fertilization treatments (p=0.0002), with a quadratic trend (p=0.0001). However, the low N was different from the high N, with the control not different than either level. Seedling pars showed differences among fertilization treatments (p=0.0001) (figure 2-4), but tap roots again had the highest starch concentration and needles the lowest.

The coefficients of variation for starch concentration showed significant differences among parts of the seedling (p=0.0001). There were no significant differences among N treatments (p=0.0926), nor for the N treatment by seedling part interaction (p=0.9831).

a)



b)



c)

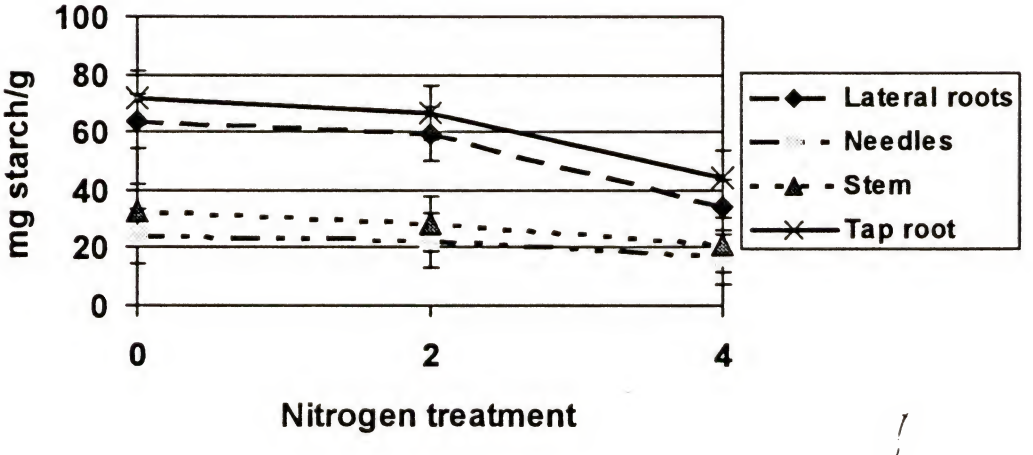


Figure 2-3. Starch concentration in bare-root seedlings: a) Among N treatments; b) Among part of the seedling; c) Among N treatments by part of the seedlings. The error bars represent standard errors.

When analyzing seedling parts, there were differences among N levels for lateral roots (p=0.0415), with a quadratic trend (p=0.0138). The needles also exhibited differences (p=0.0068), with a quadratic trend (0.0026). However, the low N level was different from the high N level with the control not different from either level. No differences were found for starch concentration in stems among N treatments (p=0.2206), and no linear (p=0.4654) nor quadratic (p=0.1164) trends were found, but Duncan's multiple range tests exhibited differences between the low N level and the other levels. No difference was found for starch concentration in tap roots (p=0.1124). However, the Duncan's multiple ranges test exhibited differences between the high N level and the other levels of N.

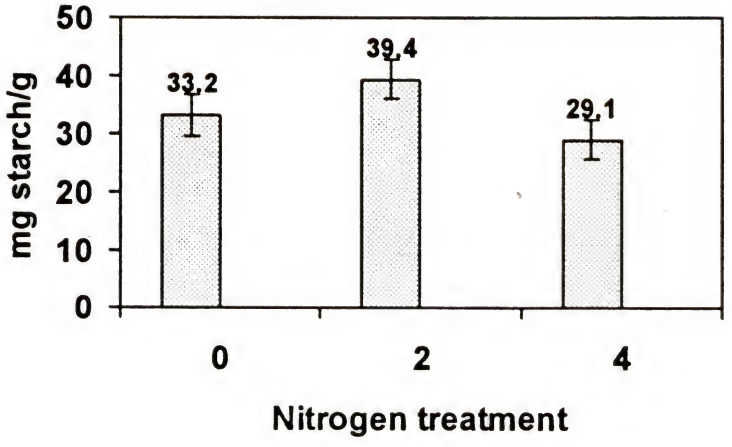
Discussion

Morphological Variables

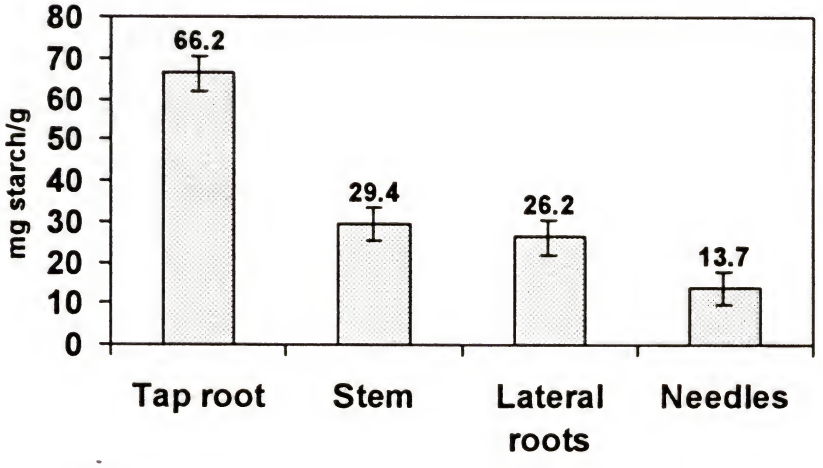
The effects of N fertilization in the nursery on morphology of pine seedlings at planting time are variable in the literature. In some studies, there are morphological changes with N fertilization (e.g., Cantin et al. 1997), but in others there is no response (e.g., Elliott and White 1994). In the present study, the high N treatments resulted in earlier bud break. This response has been observed in other species, such as *Pinus elliottii* var. *elliottii* (Irwin et al. 1998), and *Pinus banksiana* Lamb. (Cantin et al. 1997). Bud set also appeared earlier with N fertilization in *P. strobus* L. (Hinesley and Wright 1988).

In all of the three seedling types (containerized from Florida and Georgia and Florida and bare root from Florida), additional N increased some shoot-related traits. Hinesley and Maki (1980) found for longleaf pine a substantial (9 to 12%) overwinter

a)



b)



c)

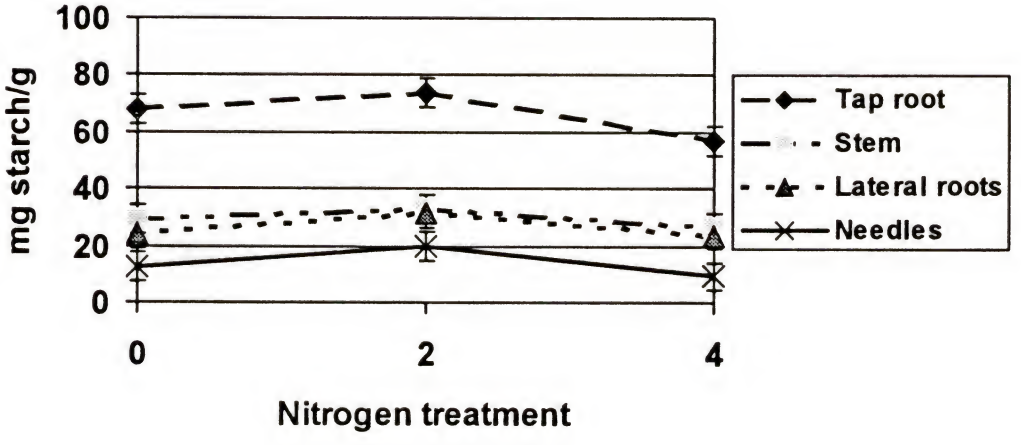


Figure 2-4. Starch concentration in containerized seedlings: a) Among N treatments; b) Among part of the seedling; c) Among N treatments by part of the seedlings.

seedling dry weight increase and a 11% increment in caliper for one-year-old longleaf pines fall-fertilized with N. About 75% of this increase was in roots and buds. Similarly, in a fall fertilization study, *P. taeda* L. seedlings under high N had greater first order lateral root numbers, stem dry weight, needle dry weight, and root dry weight (Sung et al. 1997), and shoot biomass was increased by N fertilization in *P. strobus* (Canham et al. 1996).

In the present research, no differences in total root, root part or relative root weight (as percent of total biomass) among the N fertilization treatments were found. This is contrary to the findings for roots by Hinesley and Maki (1980) for longleaf pine, and Sung et al. (1997) for *P. taeda*. Many studies also have found that shoot:root ratios are lower under low nutrient conditions (Elliott and White 1994) (*P. banksiana* Lamb., Tan and Hogan 1997, 1998), or that the fraction of biomass in roots declines as tissue N concentration increases (*P. strobus* L., Canham et al. 1996). *Pinus taeda* L. compensated for N limitations by increasing specific lateral root length and proportional biomass allocation to the lateral root system or to more biomass in the roots (Li et al. 1991, Gebauer et al. 1996).

Among others, Tan and Hogan (1998) suggest that dry weight and N partitioning may be a function of the internal status of the plant, and both shoot and root growth may have an association with whole plant N concentration. Several theoretical models include the effects of N on partitioning of plant growth into roots and shoots (Canham et al. 1996). The models predict that in order to optimize relative growth rate, allocation to roots should be a function of internal tissue N concentration (Hilbert 1990). The balance

between C and N availability is also important in determining the relative allocation of biomass to roots and shoots (Green et al. 1994). An interesting hypothetic model was proposed by Lambers et al. (1998): under high N roots produce vast amounts of cytokinins. When these cytokinins are moved to the leaves they enhance photosynthetic capacity and leaf expansion; most of the photosynthates are consumed in leaves and few sugars are sent to the roots. In a low N environment, roots produce small amounts of cytokinins. Few of those are sent to the leaves, and both photosynthetic capacity and leaf expansion are reduced. Few photosynthates are consumed in the leaves (with high concentrations of sugars), and large amounts are exported to roots for growth.

Nevertheless, in other cases, and similarly in this research, no effect of fall N fertilization or N fertilization in other seasons was found for most of the studied variables. In *P. resinosa* Ait, the shoot:root ratio was not affected after applying N fertilization (Elliott and White 1994), and Irwin et al. (1998) did not find differences in morphology among bare root *P. elliottii* var. *elliottii* (Engelm.) fall-fertilized seedlings. Along with potential variation in physiological allocation to root tissue (Canham et al. 1996), contradictory results in different fertilization experiments may result from the variability of nursery practices (Duryea 1984) and in the amounts of other nutrients provided in the nursery. Also, increased growth due to N fertilization is larger when the other nutrients are also provided in appropriate amounts (Ågren and Ingestad 1987, Waring and Running 1998).

When comparing container and bare-root seedlings, most of the variables exhibited differences. The bare-root seedlings had higher values for many of the variables because of the larger growing space available in beds, in comparison with the containers.

Responses to N fall fertilization should be focused on getting the target seedling required for the environments to be reforested. For instance, in general earlier bud break promoted by this treatment may be an advantage to gain shoot growth and overcome light competition. However, in some years the same trait may represent a disadvantage if, for example, severe late (spring) frosts are present.

Foliar Nitrogen

Bare-root and containerized seedlings had increased foliar N concentrations at high fertilization levels. This is a common response in longleaf pine (Hinesley and Maki 1980, Entry et al. 1998) and other pines such as *P. taeda* L. (Larsen et al. 1988, Sung et al. 1997), and *P. elliottii* var. *elliottii* (Irwin et al. 1998). Part of the foliar N is a component of the rubisco enzyme (ribulose 1, 5-bisphosphate carboxylase-oxygenase) that catalyzes the initial incorporation of CO_2 into sugar, and that alone can account for over 20% of leaf N in some species (Evans 1983, Margolis and Brand 1990). The extra supply of N accumulated in foliage is advantageous, because recently planted seedlings must rely on a supply of stored nutrients until they get established (Landis 1985). For example, 50 to 90% of the N and P needs are supplied from senescent foliage (Landsberg and Gower 1997).

The foliar N levels reached in the present research (e.g., the maximum mean was 1.11% for high N in bare-root seedlings) were far from the optimum levels (1.7 to 2.3%)

recommended for conifers by Duryea and McClain (1984) and Landis et al. (1989). Also, among the seedling types different traits reached maximum values at different N levels. This coincides with the findings by van den Driessche (1980) for *Pseudotsuga menziessii* (Mirb.) Franco, where shoot height, shoot and root dry weight, and shoot:root ratio increased at different rates with increasing N.

Foliar Phosphorous and P:N Relationships

Reich and Schoettle (1988) hypothesize that since positive, neutral and even negative relationships between leaf N and net photosynthetic capacity have been demonstrated in different species, the relative use efficiency of N in photosynthesis and subsequent growth are directly dependent upon the sufficient availability of other nutrients, particularly P and that a P:N ratio below 0.10% to 0.14% in *P. strobus* L. results in insufficient P for efficient N use. In the present experiment the average P:N ratios for all the N treatments were higher than 0.1 for both bare root and containerized seedlings. Also the P concentration was higher than the 0.08% referred by Mills and Jones (1996) for adult longleaf pines, so P may not be limiting growth.

The two variables that responded to N treatment in containerized seedlings from Florida, also showed a quadratic trend. If P is not limiting, perhaps other nursery factors are limiting growth at high N, although the N contents are not at toxic levels. Other limiting factors may include the limited volume for root growth for the containerized seedlings, particularly considering that P levels in containerized seedlings were three times higher than those in bare root seedlings.

Phosphorous concentrations may also have variable effects on growth. For example, in *P. serotina* Michx seedlings under low P, both the total dry weight and the root:shoot ratio decreased, in comparison with the control (Topa and Cheeseman 1992). However, in *P. pinaster* Aiton seedlings, the P treatments had no effect on biomass (Conjeaud et al. 1996).

Carbohydrates

Shoots had lower concentrations than roots, and in bare root seedlings, lateral roots and tap root starch concentrations were negatively correlated with the N treatment. Moderate nutrient deficiencies may reduce growth more than photosynthesis (Airovich and Cresswell 1983). However, new growth requires inputs, and a reduction in nutrient availability will result in starch accumulation, while an improved availability may reduce the accumulation of reserves (Waring et al. 1984). For instance, in *P. taeda* L. as N supply increased, foliar and root starch concentrations decreased, while hexose concentration increased (Green et al. 1994). Also, foliar starch concentration increased when N or water were limiting in *P. contorta* var. *latifolia* seedlings (Stewart and Lieffers 1993). This suggests that seedlings under N stress may allocate a higher percentage of fixed carbon to storage and less to transport for metabolic functions (Green et al. 1994). Also the synthesis of metabolically expensive, carbon rich compounds like lignin and phenolics is increased under high light and low N availability (Margolis and Brad 1990).

There have been contradictory findings in N effects because of the influence of other factors. In a study on the effects of CO_2 and N on longleaf pine seedlings, Entry et al. (1998) found that with high N levels, there was an increment of starch and other

carbohydrates in roots and fine roots. They also found that in low N levels, starch concentration in tap roots was greater, at ambient CO_2 levels, in comparison with twice the ambient CO_2 levels.

Sugar accumulation is favored by anything that favors photosynthesis (Marshall 1985). However in the present study, sugar concentration of the stem was negatively related to N treatment. Sugar concentration responses are more variable. In *P. taeda* L., hexose foliar and root sugar concentrations increased with increasing N levels (Green et al. 1994). In contrast, in the same species, Sung et al. (1997) found that sugar metabolism was not affected by N fall fertilization. The different responses among sugars and starches can be expected, because sucrose and starch are enzymatically interconvertible. High sucrose concentrations favor the synthesis of starch, and low concentrations favor starch breakdown (Johnson and Cline 1991).

Variability in starch and sugar concentrations during different seasons has been shown for other species, with the maximum occurring in the late winter, immediately before new growth time in spring, as in *Pinus elliottii* var. *elliottii* adult trees for all type of vegetative parts (Gholz and Cropper 1991), *Pseudotsuga menziessii* (Mirb.) Franco in twigs and needles (Webb and Kilpatrick 1993) and *Pinus taeda* L. (Adams et al. 1986, Birk and Matson 1986). Even the relationship between N and starch concentration may change from one season to another. For adult *P. taeda* L., Birk and Matson (1986) mention a positive relationship between starch and N concentration during the dormant season and a negative relationship during the growing season.

Conclusions

Nitrogen concentration in needles increased with high N treatments, and this may represent an extra supply for seedlings after planting, particularly in poor soils. Phosphorous was not limiting, and even the P concentration in containerized seedlings was three times that of bare root seedlings.

In bare root seedlings, tap root and lateral roots decreased their starch concentration in response to increased N levels, evidence that in a poor N environment (control), the seedling tended to invest more in accumulating reserves in roots. Overall, the seedlings tended to store more starch in roots than in shoots. The lack of differences in containerized seedlings, may be related to a earlier spring growth or to the limiting growing space.

Very few morphological variables responded to N fall fertilization. However, timing of spring bud burst was affected in all seedling types and this may be crucial resulting in a growth advantage in a competitive environment. Considering the extra N storage and the ability to induce earlier bud break, fall fertilization is recommended. However, additional research into fertilization (both in the fall and during the growing season) and at higher N levels (e.g., reaching a 2% of foliar N) is recommended.

CHAPTER 3
LONGLEAF PINE SEEDLING PERFORMANCE AFTER FALL FERTILIZATION

Introduction

Longleaf pine (*Pinus palustris* Mill.) has become the focus for biodiversity, conservation and restoration in the southeastern United States. However, there are many obstacles to overcome to attain longleaf pine reforestation success. These include inconsistent seed production, sensitivity to competition and root exposure during lifting. A prolonged time period before growth in the field (grass stage) makes not very attractive this species for reforestation among land owners. Despite important advances in nursery and reforestation knowledge and technology, reforestation failures are common, particularly when the planting site is a stressful environment or during years with drought.

The size of longleaf pine seedlings has been positively correlated to first and even third year survival at the planting site (e.g., Brown 1964, Kais 1980, White 1981). To perform well, a containerized seedling must have a caliper larger or equal to 0.44 cm, abundant secondary needles, and high root and shoot weight (Barnett 1984a). For the case of bare-root stock, a root collar diameter larger or equal to 1 cm, at least six primary lateral roots (larger or equal than 2 mm in diameter), a fibrous root system, 25% or more of total feeder root ectomycorrhizae, a tap root larger or equal to 15 cm, and a well developed white bud are indicators of good performance (Wakeley 1948, White 1979, May 1985, Cordell et al. 1990). There is information on longleaf pine survival under

different nursery treatments and field conditions, including cultural treatments. However, the information on how the seedlings grow in the field and how they allocate biomass is scarce.

The present chapter is the second part of a research project that analyzed the effects of nursery nitrogen (N) fertilization on the morphology and carbohydrate, N and phosphorous (P) concentrations of containerized and bare-root longleaf pine seedlings. This chapter presents first year survival and growth after planting and investigates the relationship of field performance to initial seedling morphological and physiological characteristics. The objective of this research was to analyze how seedlings from different fertilization treatments grow in the field and to investigate correlations among morphological (e.g., stem diameter and root biomass) and physiological (e.g., starch and sugar concentration in needles, stem, tap root and lateral roots, and foliar N concentration) variables with field performance at the planting site.

Methodology

Nursery

Bare-root and containerized seedlings were compared. The bare-root seedlings were produced at a nursery in northern-central Florida. All the seedlings were produced with seed from the same lot, collected by International Paper Co. longleaf pine forests near Bainbridge, Georgia (Seed lot ICHILL93). Seed was sown in October 1996, at a seedling density of 100/m^2. The needles were clipped and the roots pruned. Fertilization and irrigation were provided as scheduled for nursery production. The seedlings were lifted in February 1998 then stored at 2°C for six weeks.

The experimental design for bare-root seedlings consisted of a factorial, randomized complete block design, with five blocks selected randomly along five contiguous beds. In each block three fall N fertilization treatments: zero, two, and four applications of 168 kg/ha of ammonium nitrate (NH_4NO_3) every other week were randomly assigned, and named respectively, no N, low N and high N. Each experimental plot was 5 m in length with a 0.6 m wide buffer zone between the treatments. Each experimental plot had around 600 seedlings (1,800 by block) for a total of approximately 9,000 seedlings. At lifting, a sixth block for planting was formed with seedlings from each of the other five blocks.

In the same nursery, containerized seedlings were produced using polyethylene trays with 96 cavities ($450/m^2$) and a cell volume of 94 cm^3. The growing media was a mix of peat moss, vermiculite and perlite and seed was sown in March 1997. No clipping was practiced, and fertilization and irrigation were provided as scheduled for the production of longleaf pine at the nursery. The experimental design was a factorial, randomized complete block design, with six blocks, where three fall N fertilization treatments were randomly assigned: zero, two and four applications of 100 ppm of N, applied as ammonium nitrate solution. Every block had three experimental plots. Each experimental plot had four trays for a total of 72 trays and approximately 6,912 seedlings.

The other containerized seedlings were grown at a nursery in southern Georgia in polyethylene multi-pot seedling trays with 40 cavities each ($571/m^2$) and a cell volume of 107 cm^3. Growing media was a mix of peat moss, vermiculite and perlite. The seedlings were not needle-clipped and were irrigated and fertilized as scheduled for nursery

production. The N treatment was the same as for the other containerized seedlings. There were 12 trays per experimental plot, or 480 seedlings, for a total of approximately 8,640. Before planting, the seedlings were transported to Florida and kept in a greenhouse for four weeks.

Morphological Variables

Thirty-two morphological variables were measured or calculated for 144 bare-root seedlings, and 29 variables for 144 of each type of the containerized seedlings (n=432). The relative growth rate (RGR) was calculated for parts of the seedling and for the total dry weight according to the following model (Kozlowski and Pallardy 1997):

$$RGR = (\ln W_2 - \ln W_1) / (T_2 - T_1),$$

where W_2 is the dry weight after one year of growth, W_1 the dry weight at planting time, T_2 is one year, and T_1 is zero.

Physiological Variables

Sugars and starches were determined with the anthrone method, as described by Johnson (1995) for needles, stems, lateral roots and tap roots for 144 bare-root seedlings and 144 containerized seedlings from Georgia (288 in total). Foliar N was also determined with the micro Kjeldahl method (Bremner and Mulvaney 1982) for 288 needle samples (see chapter 2).

Outplanting

Seedlings were planted at a clearcut at the University of Florida's Austin Cary Memorial Forest in March 1998 on pomona soil series soils (USGS 1999), with 1,107 mm of precipitation during the first year, little rain during spring and fall after planting and a

mean for of 1,269 mm for the past ten years (University of Florida, unpublished data). Five hundred and forty seedlings were planted (180 bare root, 180 containerized from the nursery in Florida, and 180 containerized from the nursery in Georgia) in a randomized complete block design with six blocks. The three nursery fall N fertilization treatments were randomized within each block and planted in rows of ten seedlings each. In May, glyphosate herbicide was applied to control the competing vegetation. One year after the planting, survival was recorded and two or three seedlings from each treatment were harvested, measured, dried and weighed, to conduct a second morphological analysis (variables are shown in table 3-1).

Statistical Analyses

Two statistical approaches were used: 1) Performance of each type of seedling was analyzed individually for fertilization treatment differences; 2) Bare-root and containerized seedlings were pooled and it was assumed that the fertilization treatments applied to each seedling type provided an equivalent amount of N to seedling growth. The following models were the same for morphology and survival. In the first approach:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk},$$

with μ representing the overall mean, α the effect of the nursery N treatment (fixed effect), and β the effect of blocks (random effect). In the second approach:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \varepsilon_{ijkl},$$

where μ is the overall mean, α is the effect of seedling type (fixed effect), β the effect of nursery N treatment (fixed effect), and γ the effect of blocks (random effect). In both

approaches, the effects of interactions are represented by the combinations of letters, and ε is the random error term $(0, \sigma^2)$.

Analysis of variance was conducted using SAS for microcomputers, (v. 6.12): proc mixed, and running orthogonal contrasts (coefficients taken from Montgomery 1997). The degrees of freedom were computed with the Satterthwaite option (ddfm=satterth) (Littell et al. 1999). Survival data were normalized using the arc sin function (arc sin of square root of survival expressed as percent/100). Duncan's multiple range test (proc glm) was also used to differentiate among means. Correlations between morphological and physiological variables of nursery seedlings (independent variables) and survival and morphological variables of one year old seedlings (dependent variables) by N treatment and block number were examined using Pearson's correlation coefficient (proc corr). To compare the biomass of seedlings at planting time with biomass at age one (pooled and individualized analyses), proc mixed was used. Time of bud break in the spring was analyzed non-parametrically, with a Chi square (χ^2) test, using proc freq.

To control the table-wide type I error rate the Bonferroni inequality was applied, as described by Rice (1989). If $p_i \leq \alpha / (1 + k - i)$, then the p_i-value was table-wide significant. p_i = i-th value, $\alpha = 0.10$, k=total number of p-values, and i-successive number. When the table-wide significance is not mentioned in the results, data are only significant at the individual p-level, without the Bonferroni correction.

Results

Survival

Survival was independent of fertilization levels across all of the seedling types ($p>0.15$). However, the different types of seedlings responded differently to N availability ($p=0.0299$). Survival of bare-root seedlings was 55% and 56.7% for no N and low N, but was reduced to 36.7% at high N. Survival in containerized seedlings from Georgia (50%, 53.3%, 51.7% for levels 0, 2, and 4) and containerized seedlings from Florida (88.3%, 86.7%, and 95% for levels 0, 2, and 4), was not affected by N level.

Morphological Variables

One year after planting, no variable showed differences among the fertilization treatments (Table 3-1). Nursery N treatment did not affect the timing of spring bud burst one year after planting (data not shown, Chi square, $p=0.714$).

No morphological variables were affected by fertilization treatments in the Florida or Georgia containerized seedlings (Tables 3-2 and 3-3). Bud break timing was also unaffected by N treatments (Chi square, $p=0.5550$).

For the overall analysis comparing seedling types, 11 out of 14 measured variables exhibited differences among seedling types (Table 3-4). Bare-root seedlings were taller with a bigger stem diameter and had greater root and shoot weight. Timing of bud break in the spring after planting was influenced by seedling type (Chi square, $p=0.0070$), but not by the N treatment (Chi square, $p=0.7220$). Approximately 37% of bare root seedlings, 25% of containerized seedlings from Georgia, and 11% of containerized seedlings from Florida still had no bud break in March, one year after planting, and

Table 3-1. Bare-root seedlings morphology after one year of planting date.

Variable	Fertilizer applications 0	2	4	p-value	p-lin	p-quad
Shoot						
stem diameter (mm)	19.9	22.2	19.8	0.1685	0.9343	0.0605
bud diameter (mm)	13.4	14.8	13.1	0.4227	0.8048	0.2028
needle length(cm)	40.4	42.3	36.3	0.1326	0.1789	0.1257
stem length (cm)	4.0	4.4	3.5a	0.2305	0.3884	0.1346
Root						
root length(cm)	55.0**ab**	65.8**a**	48.3**b**	0.0415	0.4300	0.0159
tap length (cm)	44.7	50.1	43.4	0.4396	0.7644	0.2129
Dry weight (g)						
needle	22.3**ab**	25.9**a**	14.0**b**	0.0643	0.1126	0.0748
stem	6.9	7.8	5.5	0.3226	0.3737	0.2191
shoot	29.2**ab**	33.8**a**	19.6**b**	0.0905	0.1456	0.0917
tap	14.6	17.1	15.4	0.5748	0.7346	0.3247
lateral root	19.2	19.9	9.8	0.0979	0.0734	0.2095
root	33.8	37.1	25.3	0.2486	0.2489	0.2182
total	63.0	70.9	44.9	0.1379	0.1785	0.1323
S:R ratio	0.89	0.93	0.78	0.4368	0.3045	0.4380

p-value is the p-value (from proc mixed) for N treatments, p-lin is the p-value for the linear contrast, p-quad is the p-value for the quadratic contrast (both from proc mixed). The rows with values with different letter (a, b) exhibited differences ($p<0.05$) according to the Duncan's multiple ranges test (based in proc glm). No variable was table-wide significant.

21.15%, 27.78%, and 23.53% of all seedlings with no N, low N and high N, respectively, had not broken bud. Shoot:root ratio was not different among seedling types. No variable exhibited differences among N treatments, but in two cases the interaction of fertilization treatment and seedling type yielded differences: root length ($p=0.0093$), and needle dry weight ($p=0.0397$). In bare-root seedlings, the largest root length was reached

at low N, but in the containerized seedlings from Georgia and Florida it was reached at high N. In bare-root seedlings, the high N fertilization corresponded to the lowest needle dry weight, but in the containerized seedlings from Georgia and Florida, high N corresponded to the highest needle dry weight.

Table 3-2. Containerized seedlings from Georgia morphology after one year of planting date.

Variable	Fertilizer applications 0	2	4	p-value	p-lin	p-quad
Shoot						
stem diameter (mm)	18.8	16.2	19.2	0.3061	0.8739	0.1356
bud diameter (mm)	13.2	11.1	11.5	0.5689	0.4296	0.4850
needle length (cm)	35.9	31.2	33.9	0.6706	0.7168	0.4212
stem length (cm)	2.6	2.6	2.6	0.9725	0.9042	0.8451
Root						
root length (cm)	41.9	34.8	47.5	0.2080	0.4292	0.1135
tap length (cm)	38.2	33.0	42.8	0.3089	0.4824	0.1738
Dry weight (g)						
needle	9.4	8.4	11.6	0.6893	0.5755	0.5200
stem	4.8	3.6	4.2	0.6976	0.6926	0.4613
shoot	14.2	12.0	15.8	0.7300	0.7364	0.4808
tap	10.7	7.0	9.9	0.4476	0.7864	0.2233
lateral root	10.4	7.3	7.9	0.7569	0.5707	0.6381
roots	21.2	14.4	17.8	0.6226	0.6315	0.4045
total	35.4	26.3	33.6	0.7175	0.8834	0.4315
S:R ratio	0.72	0.97	0.94	0.0889	0.0719	0.1918

p-value is the p-value(from proc mixed) for N treatments, p-lin is the p-value for the linear contrast, p-quad is the p-value for the quadratic contrast (both from proc mixed). The rows with values with different letter (a, b) exhibited differences according to the Duncan's multiple ranges test (based in proc glm). No value was table-wide significant.

Table 3-3. Morphology of containerized seedlings from Florida, one year after planting.

Variable	Fertilizer applications 0	2	4	p-value	p-lin	p-quad
Shoot						
stem diameter (mm)	16.9	18.1	18.0	0.5792	0.4103	0.5223
bud diameter (mm)	13.9	14.2	14.0	0.9859	0.9622	0.8738
needle length (cm)	36.7	39.1	39.7	0.6546	0.3829	0.7788
stem length (cm)	2.6	2.7	2.9	0.7306	0.4398	0.9779
Root						
root length (cm)	5.8	46.2	54.1	0.3223	0.1849	0.4788
tap length (cm)	40.4	39.0	49.7	0.1511	0.1204	0.2370
Dry weight (g)						
needle	10.0	14.5	15.3	0.2748	0.1379	0.5420
stem	3.4	4.0	4.3	0.7181	0.4337	0.8925
shoot	13.4	18.5	19.6	0.3162	0.1570	0.5920
tap	9.5	10.4	11.5	0.5910	0.3141	0.9462
lateral root	8.4	11.7	10.7	0.7073	0.5679	0.5532
root	17.9	22.0	22.2	0.6935	0.4557	0.6914
total	31.3	40.5	41.8	0.5304	0.3119	0.6506
S:R ratio	0.80	0.88	0.90	0.2915	0.1335	0.6561

p-value is the p-value (from proc mixed), p-lin is the p-value for the linear contrast, p-quad is the p-value for the quadratic contrast (both from proc mixed). The rows with values with different letter (a, b) exhibited differences according to the Duncan's multiple ranges test (based in proc glm). No value was table-wide significant.

Correlations Between Morphological and Physiological Variables in the Nursery Phase and Field Performance Variables

Fifteen combinations had significant correlations, but no one was significant at the table-wide level (Table 3-5). At the individual p-level, only root length in the nursery exhibited significant correlation with survival. Root length was correlated with 7 other performance variables, including the total dry weight of seedlings one year after planting.

Table 3-4. Comparison among seedling types and N treatments.

Variable	Seedling type			p-see	N treatment			p-nit	p-lin	p-quad	p-int
	BR	C (Ga)	C (Fl)		0	2	4				
Shoot											
diameter (mm)	20.7**a**	18.0**b**	17.7**b**	**0.0020**	18.5a	18.9a	19.0a	0.8795	0.6274	0.8888	0.0992
bud diameter (mm)	13.8**a**	11.9**b**	14.1**a**	0.0468	13.5a	13.4a	12.9a	0.8853	0.6474	0.8689	0.5599
needle length (cm)	39.7**a**	33.6**b**	38.5**a**	**0.0033**	37.6a	37.6a	36.7a	0.9223	0.7290	0.8468	0.1825
stem length (cm)	4.0**a**	2.6**b**	2.7**b**	**0.0001**	3.1a	3.3a	3.0a	0.4971	0.7736	0.2618	0.3988
Root											
root length (cm)	55.9**a**	41.2**c**	48.7**b**	**0.0004**	47.0a	48.9a	49.7a	0.7451	0.4579	0.8544	**0.0093**
tap length(cm)	45.9**a**	37.9**b**	43.1**ab**	0.0673	41.0a	40.7a	45.1a	0.3675	0.2406	0.4252	0.2068
Dry weight (g)											
needle	20.7**a**	9.7**b**	13.3**b**	**0.0001**	13.8a	16.3a	13.6a	0.4179	0.9157	0.1885	0.0397
stem	6.8**a**	4.1**b**	3.9**b**	**0.0001**	4.9a	5.1a	4.7a	0.7828	0.6791	0.5714	0.2949
shoot	27.5**a**	13.8**b**	17.2**b**	**0.0001**	18.8a	21.4a	18.2a	0.4843	0.8570	0.2341	0.0557

Table 3-4--Continued.

Variable	Seedling type			p-see	N treatment			p-nit	p-lin	p-quad	p-int
	BR	C (Ga)	C (Fl)		0	2	4				
Dry weight (g)											
tap	15.7**a**	9.2**b**	10.5**b**	**0.0001**	11.5a	11.5a	12.3a	0.8095	0.5834	0.7293	0.3884
lateral roots	16.3**a**	8.4**b**	10.3**b**	**0.0026**	12.5a	13.0a	9.4a	0.2548	0.1917	0.3054	0.1901
root	32.0**a**	17.5**b**	20.7**b**	**0.0001**	24.0a	24.5a	21.7a	0.6732	0.4939	0.5676	0.2570
total	59.5**a**	31.3**b**	37.9**b**	**0.0001**	42.8a	45.9a	39.9a	0.6104	0.6408	0.3794	0.1257
S:R ratio	0.87a	0.88a	0.86a	0.9220	0.80**b**	0.93**a**	0.88**ab**	0.0960	0.1985	0.0821	0.1558

BR=bare-root, C(Ga)=containerized from Georgia, C(Fl)=containerized from Florida, dw=dry weight, p-see=p-value (proc mixed) for seedling type, p-nit=p-value (proc mixed) for N treatment, p-lin and p-quad are the p-values (proc mixed) for linear and quadratic contrasts for N treatments, p-int=p-value for the interaction seedling type by N treatment (proc mixed). The values with different letters in each sub-row are different, according to the Duncan's multiple ranges test (proc glm). Bolded values were table-wide significant after the Bonferroni inequality.

Table 3-5. Correlations among nursery morphological and physiological variables, and performance variables after one year in the field, bare-root seedlings.

Variables	r	p-value
Survival* with root length+	0.5446	0.0194
Stem diameter* with root length+	0.5452	0.0193
Stem length* with secondary needles number+	0.5107	0.0303
with primary needles number+	0.6451	0.0038
with root length+	0.6450	0.0039
with stem length+	0.4971	0.0358
Stem dry weight* with primary needles number+	0.5783	0.0119
with root length+	0.6643	0.0026
Needle length* with new needles number+	0.4713	0.0484
Needle dry weight* with root length+	0.6154	0.0066
Shoot dry weight* with primary needles number+	0.4803	0.0436
with root length+	0.6364	0.0045
Tap dry weight* with shoot:root ratio+	0.5628	0.0150
Lateral roots dry weight* with cut roots dry weight+	-0.4679	0.0502
Total dry weight* with root length+	0.5263	0.0248
Shoot:root ratio* with sugar concentration in stem+	0.4813	0.0431

*at age one, +at planting. r = Pearson's correlation coefficient. No correlation was significant at the table-wide level, after the Bonferroni inequality.

For the containerized seedlings from Georgia, 11 correlations were significant at the individual level, but none at the table-wide level (Table 3-6). In the case of the containerized seedlings from Florida, 17 combinations were correlated at the individual level, but only two at the table-wide level (Table 3-7): Seedlings with larger primary needle lengths at planting time had larger calipers one year after planting date, and seedlings with larger shoot lengths at planting time had larger calipers one year later.

Table 3-6. Correlations among nursery morphological and physiological variables and performance variables at age one, containerized seedlings from Georgia.

Variables	r	p-value
Bud diameter* with secondary needles number+	0.4649	0.0519
with starch concentration in tap+	0.4672	0.0506
with secondary needles dry weight+	0.4735	0.0471
with starch concentration in stem+	0.4839	0.0419
Stem length* with sugar concentration in tap+	0.4774	0.0451
Needle length* with secondary needles dry weight+	0.6056	0.0077
with shoot dry weight+	0.6155	0.0066
with total dry weight+	0.5797	0.0117
with starch concentration in lateral roots+	0.6126	0.0069
Needle dry weight* with bud number+	-0.4721	0.0479
Shoot dry weight* with primary needles maximum length+	-0.5670	0.0141
with starch concentration in needles+	-0.5438	0.0197
Lateral roots dry weight* with sugar concentration in tap root+	0.5704	0.0134
Total dry weight* with sugar concentration in tap+	0.4622	0.0535

*at age one, +at planting. r = Pearson's correlation coefficient. No correlation was table-wide significant.

Growth

In the analysis by seedling type almost every considered variable had noticeable growth. For example, the bare-root seedlings grew 47% in caliper, 111% in stem length, and 150% in tap-root length, and their shoot:root ratio decreased 37%. Except for bud diameter (p=0.0607) and secondary needle length (p=0.9773) both at high N, all the variables increased significantly over the year (Table 3-9).

Table 3-7. Correlations among nursery morphologic variables and performance nursery variables at age one, containerized seedlings from Florida.

Variable	r	p-value
Survival* with lateral roots number+	-0.6171	0.0064
with primary needles dry weight+	0.5252	0.0252
with shoot dry weight+	0.4704	0.0488
Stem diameter* with primary needles number+	0.5929	0.0095
with primary needles maximum length+	0.8141	**0.0001**
with stem length+	0.6274	0.0053
with primary needles dry weight+	0.6192	0.0061
with tap dry weight+	0.4717	0.0481
with root dry weight+	0.4856	0.0411
with shoot length+	0.7555	**0.0003**
with sturdiness coefficient+	0.4971	0.0358
Stem length* with primary needles dry weight+	0.4630	0.0530
Stem dry weight* with lateral roots dry weight+	0.5493	0.0182
Needles maximum length* with bud length+	0.4535	0.0587
with new needles number	0.5021	0.0337
Tap length* with primary needles dry weight+	0.4779	0.0449
Root length* with primary needles maximum length+	0.4774	0.0451
with primary needles dry weight+	0.6367	0.0045
Shoot:root ratio* with sturdiness coefficient+	0.4657	0.0514

*at age one. +at planting. r = Pearson's correlation coefficient.
The bolded correlations were significant at a table-wide level (Bonferroni inequality).

For containerized seedlings from Georgia, the stem diameter increased by 64%, tap-root length by 381%, the stem length by 100%, and the shoot:root ratio decreased 37%. Most of the variables had differences (p-values from 0.0001 to 0.0237). Only bud diameter did not change over the time (p=0.1569).

The containerized seedlings from Florida had a 77% increase in diameter, 281% increase in tap length, and only a 13% increase in stem length (Table 3-8). The majority

Table 3-8. Longleaf pine seedlings size at planting time and one year later.

Variable	Bare root seedlings			Containerized seedlings from Georgia			Containerized seedlings from Florida		
	0	1	p-value	0	1	p-value	0	1	p-value
Shoot									
diameter (mm)	14.1	20.7	**0.0001**	11.0	18.0	**0.0001**	10.0	17.7	**0.0001**
bud diameter (mm)	11.2	13.9	**0.0001**	9.3	11.9	**0.0001**	8.6	14.1	**0.0001**
needle length (cm)	34.7	39.8	**0.0001**	35.7	33.5	**0.0001**	36.1	38.5	**0.0008**
stem length (cm)	1.9	4.0	**0.0001**	1.3	2.6	**0.0001**	2.4	2.7	0.5339
Root									
tap length (cm)	18.5	46.2	**0.0001**	7.9	38.0	**0.0001**	11.3	43.1	**0.0001**
root length (cm)	30.2	56.4	**0.0001**	22.4	41.3	**0.0001**	34.4	48.7	**0.0001**
Shoot:root ratio	1.38	0.87	**0.0001**	1.38	0.88	**0.0001**	0.90	0.86	0.4234

0=at planting, 1=after one year of planting.

of variables had differences, with highest values after one year of growth and p-values from 0.0001 to 0.0416.

Almost all of the variables increased between planting time and one year after planting. Only stem length for the containerized seedlings from Florida and shoot:root ratio for the same type of seedlings had no differences (Table 3-8). In the pooled analysis most of the variables had differences between planting time and one year later, except for secondary needles length, at no N, low N and high N, and stem length at low N (Table 3-9).

Table 3-9. Longleaf pine seedlings growth by nitrogen treatment during the first year after planting.

Variable	Fertilization:	Level 0			Level 2			Level 4		
	Year:	0	1	p-value	0	1	p-value	0	1	p-value
Shoot										
Caliper (mm)		11.7	18.5	**0.0001**	11.8	18.9	**0.0001**	11.7	18.9	**0.0001**
Bud caliper (mm)		9.2	13.5	**0.0001**	10.0	13.4	**0.0001**	9.9	12.9	**0.0001**
Stem length (cm)		1.3	3.0	**0.0001**	2.8	3.3	0.3682	1.4	3.0	**0.0001**
Needle length (cm)		35.9	37.5	0.2245	37.1	37.6	0.7041	37.6	36.7	0.4777
Root										
Root length (cm)		26.4	47.2	**0.0001**	26.4	48.9	**0.0001**	26.0	50.1	**0.0001**
Tap length (cm)		12.5	41.1	**0.0001**	12.6	40.7	**0.0001**	12.6	45.5	**0.0001**
Dry weight										
Stem dw		0.8	4.9	**0.0001**	0.9	5.1	**0.0001**	1.0	4.7	**0.0001**
Needle dw		4.2	13.8	**0.0001**	4.4	16.3	**0.0001**	4.8	13.7	**0.0001**
Shoot dw		5.1	18.7	**0.0001**	5.3	21.4	**0.0001**	5.8	18.3	**0.0001**
Tap dw		2.6	11.5	**0.0001**	2.6	11.5	**0.0001**	2.6	12.3	**0.0001**
Lateral root dw		1.8	12.4	**0.0001**	1.8	13.0	**0.0001**	1.9	9.5	**0.0001**
Root dw		4.4	24.0	**0.0001**	4.4	24.5	**0.0001**	4.5	21.8	**0.0001**
Total dw		9.5	42.7	**0.0001**	9.7	45.9	**0.0001**	10.4	40.1	**0.0001**
S:R ratio		1.19	0.80	**0.0001**	1.19	0.93	**0.0002**	1.31	0.88	**0.0001**

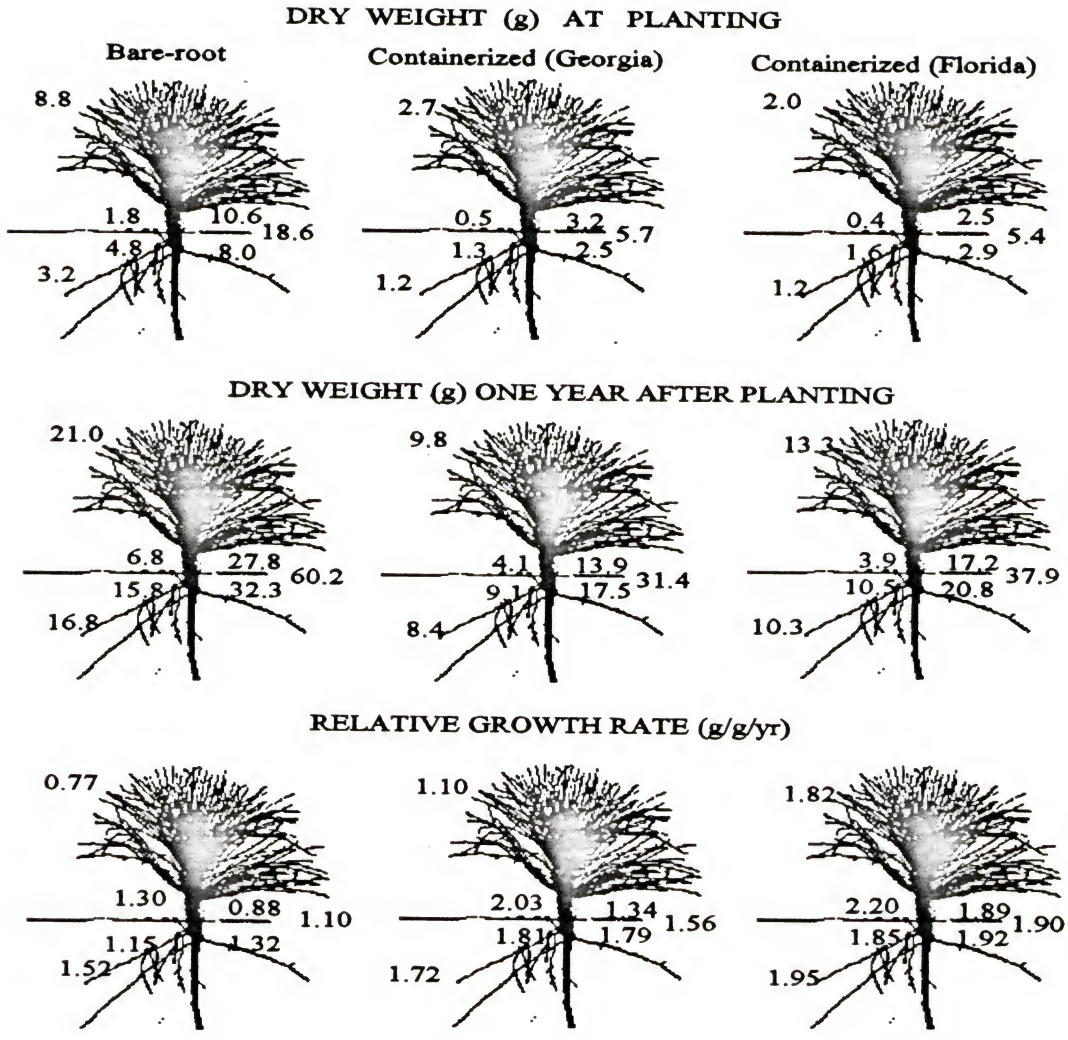


Figure 3-1. Biomass at planting time and one year later and relative growth rates (RGR) for three types of longleaf pine seedlings. The values on the left side represent needles, stem, tap root, and lateral roots. The values on the right side represent shoot, root, and whole seedling (number to right).

The relative growth rate (RGR) was larger in containerized seedlings (1.90 g/g/yr from Florida and 1.56 g/g/yr for Georgia) than in bare-root seedlings (1.10 g/g/yr).

behind bare-root seedling biomass. In all of the cases, root biomass increased more than shoot biomass. The highest RGR for bare-root seedlings was reached by lateral roots (1.52 g/g/yr). In the containerized seedlings from Georgia and Florida, the highest RGR was attained by stem (2.03 g/g/yr and 2.20 g/g/yr, respectively), and in the containerized seedlings from Florida, the highest RGR was found in stem (2.20 g/g/yr) (Figure 3-1).

Discussion

Survival

The containerized seedlings from Florida had survival superior to the bare-root seedlings. Numerous studies show the advantages of having a unexposed root and superior survival for longleaf pine containerized seedlings when compared to bare-root seedlings (e.g. Amidon et al. 1982, Ruehle 1982, Barber and Smith 1996, Compton 1996), with values from 5 to 93% for containerized seedlings, and 1 to 52% for bare-root seedlings in different conditions. Despite this, there are pros and cons for each of these seedling types.

Among the advantages of containerized seedlings is an extended planting season, improved field performance especially on adverse sites, and greater uniformity in seedling production. Some disadvantages include the necessity for more intense site preparation, the production of small sized seedlings, and the difficulty for assessing root vigor because the growing media covers the roots (Barnett et al. 1996). Also, in bare-root seedlings, the roots may be damaged during lifting (Barnett and McGilvray 1997).

(Bong.) Carr. (Benzian 1966), *Pseudotsuga menziesii* (Mirb). Franco (van den Driessche 1980a), and *Pinus elliottii* var. *elliottii* (Irwin et al. 1998)). In other species it may adversely affect survival, as in *Pinus contorta* Dougl. Ex. Loud. (Etter 1969), or not affect survival as in *Pinus elliottii* Engelm. (Gleason et al. 1990). Hinesley and Maki (1980) concluded that fertilization had no effect on longleaf pine survival, even during the second year after planting. The latter results are consistent with our results. However, contradictory findings, even within a single species, may be related to nursery practices, planting site characteristics and genetic factors. Hinesley and Maki (1980) also observed that survival and percent of seedlings emerging from grass stage were higher when fertilized at the nursery with N in the fall eight years following planting.

Morphological Variables

Bare-root seedlings had a larger initial size due to more growing space in the nursery beds, compared to containerized seedlings. In general, bare root seedlings are larger than containerized longleaf pine seedlings (Barnett et al. 1996), and larger containers produce larger seedlings. Seedlings develop better in large volume containers (Ruehle 1987, Brisette et al. 1990, Brisette et al. 1991, Barnett and McGilvray 1997). Ruehle (1982) found 93% greater root weight and 113% greater shoot weight for containers with 131 cm^3 compared to 66 cm^3 when producing longleaf pine and *Pinus taeda* L. Seedling size also influences the time when seedlings leave the grass stage, as well as their height growth in that year (Lauer 1987).

There is abundant literature in plant nutrition, but little correlating nutritional status with field performance in tree seedlings (Mattson 1997), and frequently the results are contradictory (Duryea and McClain 1984). For instance, Switzer and Nelson (1963) found a linear relationship between foliar N content at lifting and the height of *Pinus taeda* L. at age three, but in *Pinus contorta* Dougl. Ex. Loud. the correlation was poor (van den Driessche 1984). In this study, very few morphological variables after one year in the field were shown to be affected by previous fall N fertilization in the nursery. In the containerized seedlings from Georgia, the lowest N fertilization level corresponded to the minimum root length, whereas in the containerized seedlings from Florida, the highest N fertilization level had the highest root length. These different trends in carbon allocation to roots in each seedling type could be related to other growing factors in the nursery. Landsberg and Gower (1997) emphasized that there is an inadequate understanding of mechanisms controlling carbohydrate storage and utilization in tree species.

Genetic influences may also be important when considering N fertilization effects. In a greenhouse study with *Pinus elliottii* Engelm. grown under different N levels, Dewald et al. (1992) found strong family by N treatment interactions. Fast growing families exhibited more summer height growth, more flushes and later growth cessation, higher shoot:root ratios, and a higher root fibrosity at all N treatments.

Correlating Nursery Variables with Field Variables

Bare-root seedlings

Longleaf pine has a grass stage and develops a large root system. Root length was correlated with survival, stem diameter and seven other variables at age one, including

shoot and total dry weights. Similarly, Hatchell (1987) found a 1.3% increase in survival for each additional lateral root in longleaf pine seedlings with low fibrosity roots. Apparently the more roots are cut or damaged right before planting, the lower the vigor of a seedling. This is supported by the negative correlations we found between dry weight of removed roots (at planting, roots were cut at a standard 30 cm length) and lateral root dry weight after one year in field. Apparently this may be more important for species, such as longleaf pine, that develop a large root system, because Harrington and Howell (1998) found no differences in survival among treatments with altered roots in *P. taeda* L. which develops a much smaller root system. In the present study, the taller seedlings at planting developed a larger root length one year after planted. Similarly, Hatchell and Muse (1990) found a correlation between number of strong first order lateral roots and height.

For root-pruned seedlings, Hatchell and Muse (1990) also found correlations between stem diameter at planting and height one year after planting, total fresh weight and height, and number of strong, first order lateral roots and height. In the present research the stem length at age one was correlated with four different variables at planting date including stem length. Similar results for containerized seedlings were reported by Barnett (1984b).

Containerized seedlings

In the case of containerized seedlings from Georgia, there was no nursery variables that correlated with survival at age one, but the seedlings showed the highest number of correlations between physiological and morphological variables. The correlations between

carbohydrate concentrations at planting and morphological traits after one year of growth, may be evidence that the main destination of those reserves at planting is to growth of seedling parts. Tap-root sugar or starch concentrations correlated most with morphological variables one year after planting. This was expected because the study in chapter 2 found tap roots have higher carbohydrate concentrations, and because typically roots store carbohydrates to support the plant's needs (e.g., Kozlowski and Pallardy 1997, Lambers et al. 1998). Higher tap-root starch or sugar concentrations probably contributed to greater growth of the bud diameter, stem length, and lateral roots.

Containerized seedlings from Florida had the largest number of correlations between nursery variables and planting site variables, including two nursery variables correlated with survival: primary needle dry weight, and shoot dry weight. McGilvray and Barnett (1982) and Barnett (1984b) found positive correlations between survival and root weight. Stem diameter at planting was correlated to eight traits measured one year after planting. Contrary to this, Barnett (1984b) found no correlation between caliper at planting and height 1 to 3 years after planting, but found correlations for stem height at planting and height 1 to 3 years after planting. The present study showed that the higher the shoot dry weight at planting, the better the survival, and that the primary needle dry weight at planting may be a good estimator of root, tap-root length and stem length after one year in the field.

Few attempts correlating root starch, sugar, and total non-structural carbohydrates with field performance have been successful (Duryea and McClain 1984, Ritchie 1984, Johnson and Cline 1991). In the present study, needle starch concentrations at planting

were inversely correlated to shoot dry weight at age one in containerized seedlings from Georgia. This may mean that the reserves for stem growth were not taken primarily from needles.

There was high variability among seedling types (even among the two containerized types) for the nursery variables and the field performance variables that exhibited correlations. However, such variables should not be visualized statically, but dynamically, meaning that even in the presence of low quality levels for a key nursery variable, high levels in another important variable may compensate and improve the seedlings' quality and vigor. This may be a way in which different genotypes adapt to a particular environment. For instance, Hatchell and Muse (1990) found bare root seedlings with 14 or more first order lateral roots in low fibrosity root systems, or six or more first order lateral roots in high fibrosity systems both reached 80% field survival, with 60% of seedlings out of the grass stage in two years. Those authors also found that with vertical root pruning in the nursery, all the seedlings with one or the other root characteristics performed equally well in the field, regardless of the root-collar diameter, a classic indicator of performance.

As in the present research, where seedlings with larger primary needles had larger calipers one year later, the primary needles have been related to vigor traits in other pines. The reasons may be the ability of primary needles to synthesize chlorophyll in the dark (*Pinus jeffreyi* L. and *P. sylvestris* L., Schoefs and Franck 1998), and the correlation existing between number of primary needles and number of ectomycorrhizae (*Pinus contorta* Dougl. Ex Loud., Miller et al. 1988). Interestingly, in the present research,

primary needle traits (maximum length, number, and biomass) in all of the three types of seedlings at planting time appeared correlated with traits one year after planting (survival, stem and shoot biomasses, tap-root and root lengths).

Growth

There was a two-fold increment in stem length in two of the three types of seedlings. However, as expected because of the longleaf pine grass stage, roots grew more than shoots in all seedling types, resulting in a decrease in the shoot:root ratio from planting to age one. The smaller containerized seedlings had larger increments than the bare root seedlings maybe because their roots had better vigor.

Conclusions

Survival one year after planting was not improved by N fertilization in the nursery. Several of the correlations found in the present study, except for the two significant correlations at the table wide alpha, may be due to chance. However, several correlations are biologically meaningful and consistent with the literature. For example, containerized seedlings from Florida with larger primary needle lengths at planting had larger stem diameters one year later; in the literature, primary needle traits are related to vigor traits. In bare-root seedlings, survival one year after planting was correlated with root length at planting time. Survival of containerized seedlings from Florida was correlated with stem diameter and shoot dry weight.

As expected from a species with a grass stage, where the roots grow more than the shoot, longer root lengths resulted in higher survival and better vigor-related traits for bare-root seedlings. The larger initial size of bare-root seedlings when compared with

containerized seedlings was probably due to the larger growing space available in bare-root beds in comparison with containers. Better vigor in container seedlings was no common probably due to their unexposed roots and the lack of root pruning and this resulted in more field growth.

The initial morphological variables that were correlated with field performance variables in this study may be useful for operational programs. For instance, the primary needle length could be used as a criterion for culling.

The use of N fertilized containerized seedlings is recommended. However, further research on N fertilization and field performance would be beneficial. Also higher N levels applied in different seasons in the nursery should be tested. Studying the effect of higher levels of N applied in different seasons on field performance and the length of the grass stage is critical to future regeneration success.

CHAPTER 4
ARTIFICIAL REGENERATION OF LONGLEAF PINE IN GAPS

Introduction

In the southeastern United States, natural disturbances of varying types (hurricanes, forest fires, microgaps by wildlife), temporal, and spatial scales play key roles in maintaining the structure and function of longleaf pine ecosystems (Gordon et al. 1997, Palik et al. unpublished, Gillam and Platt 1999). For longleaf pine, the dominant tree species in the longleaf pine ecosystem, these and several other disturbances, among other functions, play important roles in regeneration (Wahlenberg 1946, Platt, 1998).

Forest gaps originate from disturbances, and the increased availability of light, water and nutrient levels causes new growth. Regeneration in gaps has been studied in tropical (e.g., Martínez-Ramos et al. 1988, Dirzo et al. 1992, Veenendall et al. 1996, Dalling et al. 1998, Denslow et al. 1998), temperate (e.g. Gray and Spies 1996) and even semiarid (e.g. Branch et al. 1996, 1999) environments. Forest gaps, the product of many types of disturbances, have recently become recognized as ecologically important features in temperate ecosystems (Whitemore 1989, Oliver and Larson 1990), driving the forest cycle through open, growth and closed phases (Whitmore 1989).

Longleaf pine has been largely recognized as shade intolerant, with its regeneration primarily concentrated in forest gaps (Wahlenberg 1946, Platt et al. 1988). However,

there is a lack of understanding in the ecosystem responses to overstory disturbances (Palik et al. 1997).

Landsberg and Gower (1997) emphasize the need of understanding carbon (C) allocation in naturally disturbed and managed forest ecosystems. Mitchell et al. (1996) note the need for better understanding of the community responses to disturbance regimes to improve forest ecosystem management.

The objective of this research was to study the performance (survival, morphology and growth) of bare root and containerized longleaf pine seedlings planted in the different growing conditions represented by three different gap types, and in different locations within large gaps. During the experiment (1998), the southeastern U.S. experienced a severe drought (NOAA 2000) (see discussion) that represented "extreme" conditions.

Methodology

Plant Production

Two seedling types were compared: (1) Bare-root seedlings produced in a Florida nursery and (2) Containerized seedlings produced in a nursery in Georgia. Seed was collected from International Paper Company's second-growth forest land near Bainbridge, Georgia (seed lot ICHLL93). In the bare root nursery, seed was sown in October 1996, and seedlings were lifted in February 1998. Needles were clipped, and the seedlings fertilized and irrigated as scheduled for the production of longleaf pine. Fall fertilization treatments consisted of 0, 2, or 4 applications (no N, low N and high N) of 167 kg/ha of ammonium nitrate every other week.

Containerized seedlings were grown in a media of peat moss, vermiculite and perlite in polyethylene multi-pot trays with 40 cavities each (571/m^2), and a cell volume of 107 cm^3. The needles were not clipped, and the seedlings were fertilized and irrigated as scheduled for longleaf production in this nursery. The fall fertilization treatments consisted of 0, 2, and 4 applications of ammonium nitrate solution with a N concentration of 100 ppm.

Morphological Analysis of Nursery Seedlings

Thirty-two variables for the bare-root seedlings and 29 for the containerized seedlings were measured or calculated, including shoot caliper and height and dry weights for each seedling part. Also relative dry weights were calculated by dividing each plant part dry weight (e.g. root) by the total plant dry weight.

Plantation and Experimental Design

The plantation was established in February 1998, at the International Paper Company's second-growth longleaf pine forests, near Bainbridge, Georgia. The experimental design was a factorial split plot (Figure 4-1). The whole plots were six sites (blocks) and the sub-plots were the gaps. Every site had three types of gaps: large gap (144 m diameter), small gap (28 m diameter), and forest (no gap). The gaps were opened by clearcutting in 1996 by International Paper Company. Winter and summer low intensity prescribed burns were practiced in 1997 and 1998.

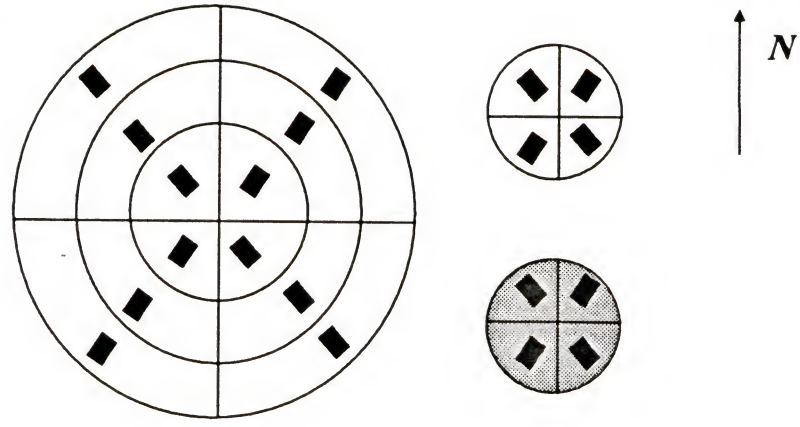


Figure 4-1. Experimental design. In each black plot containerized or bare-root seedlings (half of the plot) with three N nursery fertilization treatments were planted. The large circle depicts a large gap plot, while the small circles depict small gaps (white background) or closed forest (grey background). There are 60 seedlings per rectangle in the large gaps, and 72-90 per rectangle in the small gap and forest.

In the large gaps 10 seedlings for each stock type, nursery N treatment, cardinal direction from the center, distance from the edge, and site were planted, for a subtotal of 4,320 seedlings. In the small gaps 10 to 13 seedlings per seedling type, nursery N treatment, cardinal direction and site were planted, for a subtotal of 1,704 seedlings. In the forest, 15 seedlings per stock type, nursery N treatment, cardinal direction (NE, SE, SW, NW) and site were planted, for a sub total of 2,160 seedlings. In total 8,184 seedlings were planted in 120 plots. Containerized seedlings were planted with a planting dibble, and the bare root seedlings with a planting shovel. The roots of bare-root seedlings, were pruned with a straight edge if they exceeded 30 cm in length at planting. The distance between trees within a plot was 1 m. No herbicide or irrigation or fertilization was applied.

Survival, Morphology and Growth

Survival was recorded one year after planting. Also, two to three seedlings for each N treatment and seedling type per plot were harvested for morphological analysis after one year. Harvested seedlings were temporarily stored in a large cooler with ice and then at 2°C in a cooler. Measurements (e.g. caliper, stem length, etc.) were obtained from the seedlings. Then lateral roots, tap roots, stems and needles were separated, placed in an oven at 70°C for two weeks, and weighed. Relative growth rate (RGR) was calculated as the growth variable, according to the following model (Kozlowsky and Pallardi 1997):

$$RGR = (\ln W_2 - \ln W_1) / (T_2 - T_1),$$

where: W_2 and W_1 are the dry weights one year after planting, and at planting, and T_2 and T_1 are one year after planting (1) and the time of planting (0), respectively.

Characterization of Interactions With Understory

Competing vegetation was characterized in every plot. Plant life forms (herbs, grasses, and shrubs) with the family, the density, plant cover (%), and maximum height by life forms were recorded. Herbs included all types of non-woody vegetation except grasses.

Light

The light data were obtained from a series of studies at the same sites in Georgia conducted by McGuire et al. (in press). The following description of methodology is from McGuire et al. (in press). Hemispherical photographs were taken according to Mitchell and Whitmore (1993), with the photographs recorded on 400-speed black and white film during uniformly cloudy days or mornings prior to sunrise according to Easter and Spies

(1994). An 8 mm fisheye lens and camera assembly were leveled skyward on a tripod 1 m above the floor and oriented north. The film negatives were converted to a digital format and imported from automated digitized imaging using Hemiview® (Delta-T Devices Ltd, Burwell, Cambridge, UK). Hemiview was also used to calculate several indices of solar radiation based on canopy openness, using an index of the sun's position and projected path of movement over a six-month period. A gap light index (GLI) (*sensu* Canham 1988) was calculated for each location and gap treatment.

Statistical Analysis

The statistical model was:

$$y_{ijklmn} = \alpha_i + \beta_j + \gamma_k + \delta_l + \eta_m + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\alpha\delta)_{il} + (\alpha\eta)_{im} + (\beta\gamma)_{jk} + (\beta\delta)_{jl} + (\beta\eta)_{jm} + (\gamma\delta)_{kl} + (\gamma\eta)_{km} + (\delta\eta)_{lm} + (\alpha\beta\gamma)_{ijk} + (\alpha\beta\delta)_{ijl} + (\alpha\beta\eta)_{ijm} + (\alpha\gamma\delta)_{ikm} + (\alpha\gamma\eta)_{ikm} + (\alpha\delta\eta)_{ilm} + (\beta\gamma\delta)_{jkl} + (\beta\gamma\eta)_{jkm} + (\beta\delta\eta)_{jlm} + (\gamma\delta\eta)_{klm} + (\alpha\beta\gamma\delta)_{ijkl} + (\alpha\beta\gamma\eta)_{ijkm} + (\alpha\beta\delta\eta)_{ijlm} + (\alpha\gamma\delta\eta)_{iklm} + (\beta\gamma\delta\eta)_{jklm} + (\alpha\beta\gamma\delta\eta)_{ijklm} + \varepsilon_{ijklmn},$$

where: α is the effect of the site (random effect), β the effect of the gap (fixed effect), γ the effect of the direction (fixed effect), δ the effect of the seedling type (fixed effect), η the effect of the N treatment (fixed effect), the combinations of factors represent the respective interactions, and ε represents the random error term $(0, \sigma^2)$.

Because the large gaps were the only planting sites with a variable for distance from the center of the gap, those also were analyzed with the same type of model, excluding the factor gap type and including the factor distance, with three fixed effects levels. In another approach, every seedling type was analyzed separately, reducing the number of factors to four, with a similar model as described above.

Survival and all of the morphological variables after one year of growth were analyzed. The data were processed using SAS (Statistical Analysis system®, version 6.12), analysis of variance (proc mixed), calculating the degrees of freedom with the Satterthwaite option (ddfm=satterth) (Littell et al. 1999). Each variable was analyzed with orthogonal contrasts, linear and quadratic for the N fertilization. The coefficients were obtained from Montgomery (1997). Means and standard errors were obtained using the least squares means statement (lsmeans). For the case of the pooled analysis, after running the program with proc glm, the 3-way, 4-way, and 5-way interactions by variable with a p-value larger than 0.25 were omitted to conduct a reduced analysis with proc mixed to increase the power of the test.

Growth was analyzed with proc mixed, adding the variables time 0 (planting moment), and time 1 (one year after planting). Bud status (broken or non broken) was analyzed with the non-parametric Chi-square (χ^2) test.

To predict survival we used multiple regression models by first obtaining the Pearson's regression coefficients among survival and all of the field variables (e.g. GLI, grass cover, total cover, etc.). Light data were obtained for three of the six blocks and our models and correlations with gap light index (GLI) included the data from these three blocks. Variables were selected by magnitude of their coefficient with survival and had to be independent of other variables, to prevent multicolinearity. The residuals of every selected independent variable were also checked to see if there was some trend. A power test for survival was conduced as described by Martin (1989).

Results

Survival

In both the pooled and reduced analysis, the interaction between seedling type and gap type was significant (p=0.0059). Survival for containerized seedlings in the forest, small and large gaps was 35.1%, 23.4%, and 15.4%, respectively while survival of bare-root seedlings was 12.3%, 9.7%, and 8.9%. For both types, survival was higher under the forest canopy (23.7%) than in gaps (16.5% in small gaps, 12.2% in large gaps) (p=0.0001), and in all of the cases containerized seedlings (24.6%) exhibited better survival than bare root seedlings (10.3%) (p=0.0002). The N treatments did not have an effect on survival (p=0.5670).

Analysis by Seedling Type

Bare-root seedlings

Bud diameter, stem length, needle dry weight, stem dry weight, and shoot dry weight were larger at high N levels, and relative weight of the tap root decreased at high N levels. All followed a linear trend (Table 4-1). The shoot:root ratio was affected by the gap by direction by N treatment interaction (Figure 4-2). In the forest, only the SE direction had increased shoot:root ratio with increasing N levels. In the small gap, only the NW and SE directions at high N were larger than no N and low N. In the large gaps, no clear trends were found and most of the combinations of factors did not exhibit differences.

Allocation to all tissues was dependent on the interactions of gap type, cardinal direction, and fertilization treatment. For relative root weight a significant interaction

Table 4-1. Variables influenced by nursery N treatment in bare-root seedlings.

Variable	Number of fertilizer applications			p-value	p-lin
	0	2	4		
Bud diameter (mm)	7.6	8.4	9.0	**0.0001**	**0.0001**
Stem length (cm)	2.7	3.2	3.1	**0.0314**	**0.0466**
Stem dry weight (g)	2.1	2.7	2.6	**0.0288**	**0.0364**
Needle dry weight (g)	4.2	5.4	5.9	**0.0514**	**0.0208**
Tap root relative weight	0.41	0.37	0.37	**0.0313**	**0.0175**
Shoot dry weight (g)	6.3	8.1	8.5	**0.0449**	**0.0288**

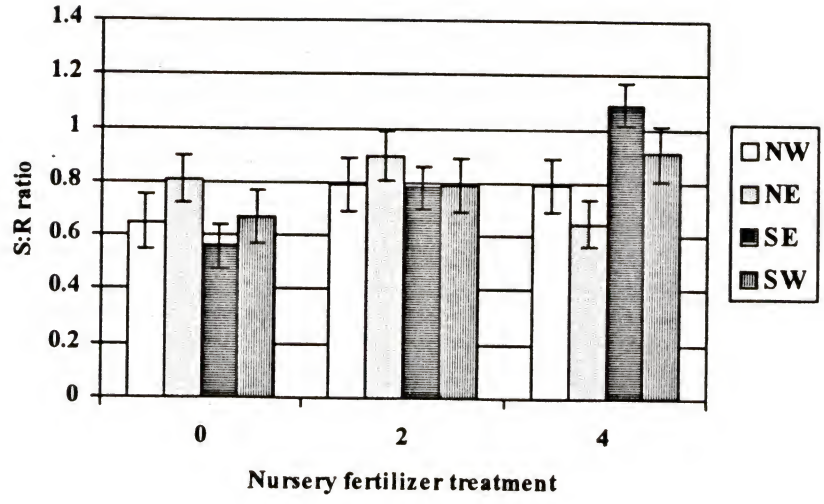
0=no nursery N applications, 2=Two fall applications of N (168 kg/ha of ammonium nitrate) in the nursery, 4=Four fall N applications in the nursery. P-lin is the p-value for the linear orthogonal contrast.

(p=0.0273) resulted from response to the aboveground allocation, also significant. In the forest, relative root weight was less at high N levels in the SE direction. In the small gaps, roots were lightest under the highest fertilization, without differences among no N and low N. No clear trends were found in the large gaps (Figure 4-3).

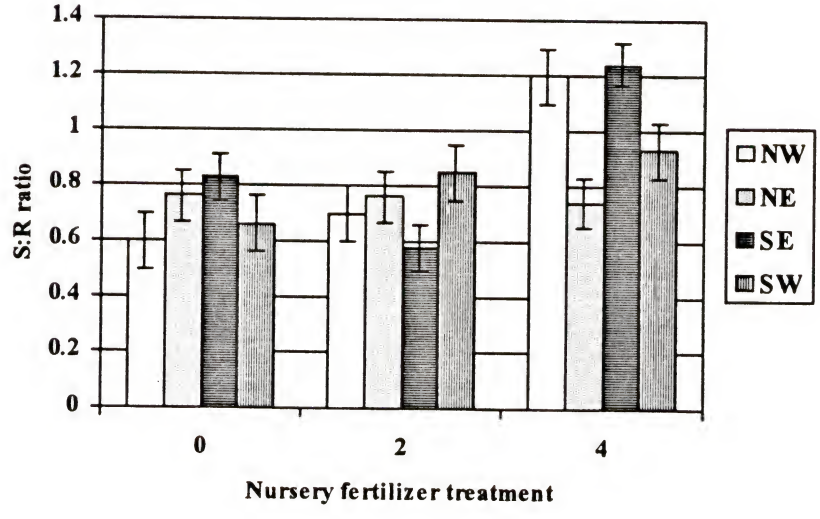
Containerized seedlings

Stem diameter was smaller in the forest (10.8 mm) when compared with small gaps (11.3 mm) and large gaps (11.7 mm) (p=0.0013). Bud diameter (p=0.0011) also was larger in the large gaps (7.5 mm), followed by the small gaps (6.7 mm), and the forest (6.1 mm).

Forest



Small gap



Large gap

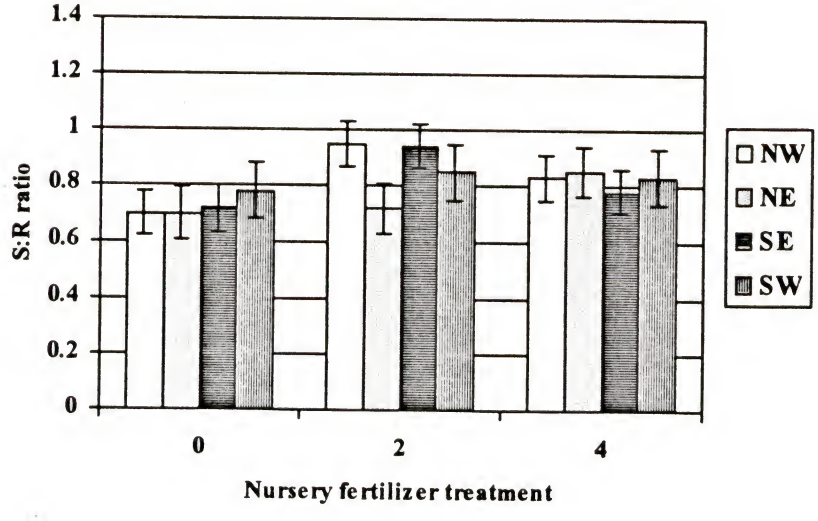
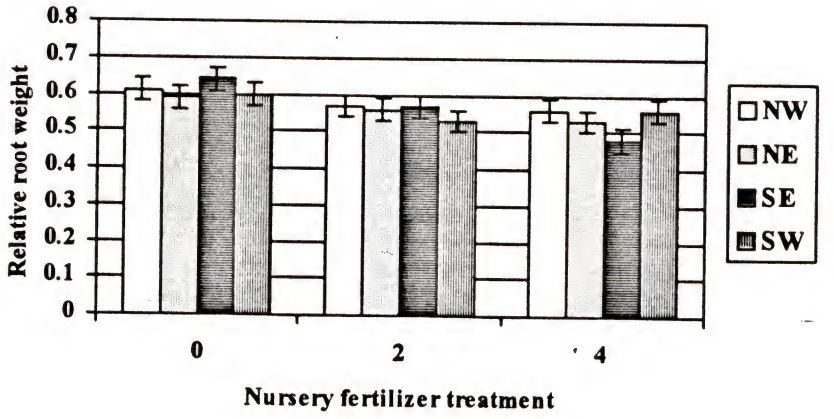
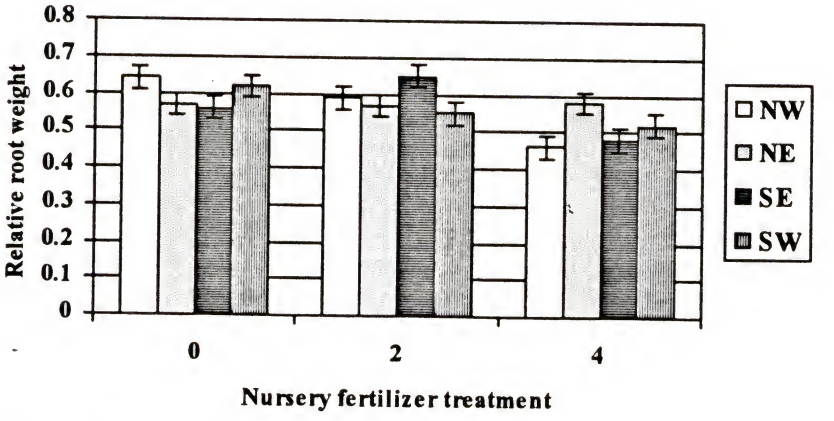


Figure 4-2. Mean (± 1 standard error) shoot:root ratio for bare-root seedlings at different gap types, N treatments and cardinal directions.

Forest



Small gap



Large gap

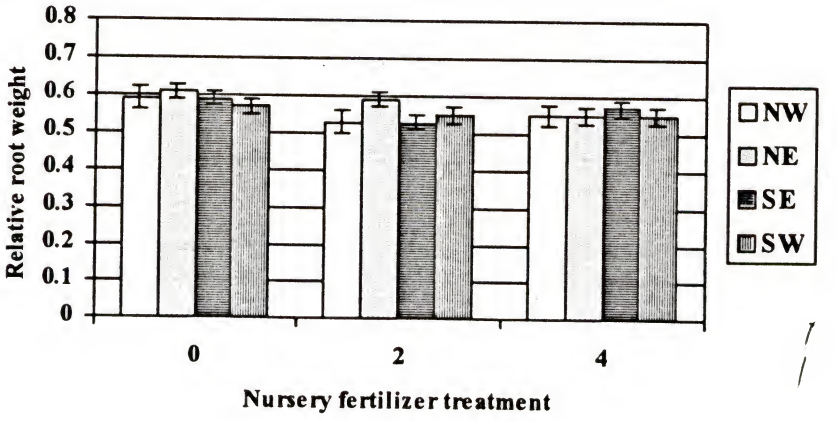


Figure 4-3. Mean (±1 standard error) relative root weight for bare-root seedlings at different gap types, directions and N treatments.

The shoot:root ratio was influenced by the interaction gap by N treatment ($p=0.0066$). In forest low N was different from no N but not from high N. In the small gaps, high N was higher than no N and low N. In the large gaps, no clear differences among N fertilizations were found (Figure 4-4).

Also the shoot relative weight was affected by the gap by N treatment interaction ($p=0.0069$). In the forest, higher levels of N corresponded to higher shoot relative weights, with no apparent trend in the large gaps among N treatments and with a positive relationship with the highest value in the small gaps at high N (Figure 4-5). The root relative weight responded also ($p=0.0069$).

The needle relative weight had significant interaction gap by N treatment interaction ($p=0.0233$). This variable was related positively to N level in the small gaps and in the forest, but not in the large gaps.

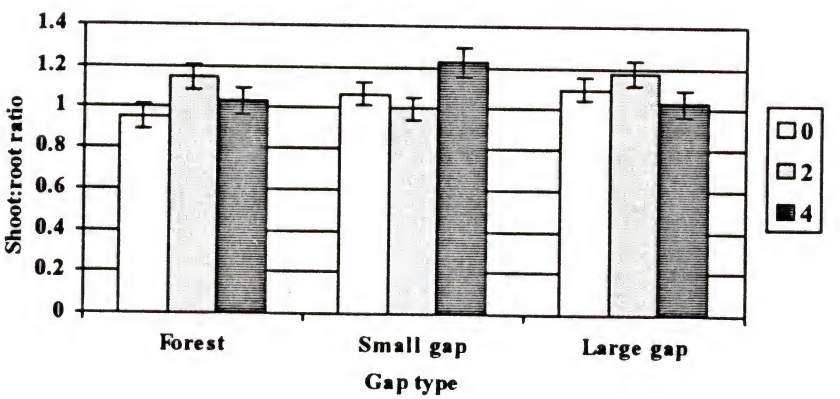


Figure 4-4. Mean (±1 standard error) shoot:root ratio for containerized seedlings at different gap types and N treatments.

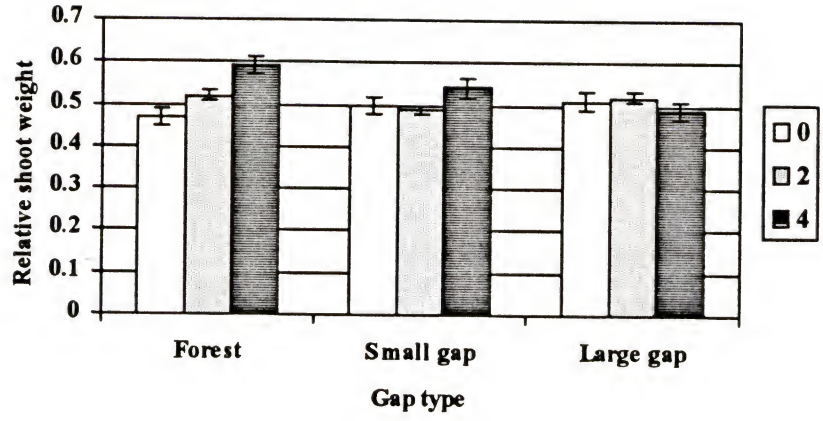


Figure 4-5. Mean (±1 standard error) relative shoot weight for containerized seedlings at different gap types and N treatments.

Bud Break and Herbivory

Non-parametric (Chi-square) analysis in a pooled comparison showed that a larger proportion of bare-root seedlings (67.5%) were breaking buds at the measurement time (in February), in comparison with 57.8% for containerized seedlings (p=0.006) (similar to chapter 3 results). Bud break timing of containerized seedlings was influenced by gap type: 70.4% for forest, 67.5% for small gaps, and 43.5% for large gaps (p=0.001). Also the proportion of needles consumed by insects and mammals was affected by gap type (p=0.007), with 89.5% of the seedlings browsed in the forest, 76.8% in small gaps, and 77.3% in large gaps.

Interaction With Subcanopy Cover

The percent cover of grasses, shrubs plus grasses, and grasses plus herbs increased with forest gap size (Table 4-2). Gap type similarly affected the herb height. No other measured competition variable was affected by gap type, direction or their interaction.

In the non-parametric analysis to look at the interaction among types of dominant competitive vegetation (herbs, grasses and shrubs) by gap type and direction, only the herbs were significantly dependent (p=0.001, Chi-square test) on gap type. The ferns were present more frequently in large gaps (42.2%) and under forest canopy (39.1%) than in small gaps (18.7%). The *Asteraceae* were more frequent in the large gaps (over 70%) than in any other gap type. *Cyperaceae* were only found in large gaps. *Convoluvulaceae* and *Rosaceae* were absent from the forest (respectively 50% and 50%; and 36.8% and 63.2% for small and large gaps), while *Lorniaceae* were present only in the forest. *Guttiferaceae* had the same frequency in the three locations. Lastly, *Fabaceae* had reduced frequencies from the large gap to the forest and small gap (50%, 36.4%, 13.6%). The most frequent dominant genus and species by gap type were: (1) In the forest, the oaks dominated among the woody vegetation, *Andropogon* and *Aristida* were dominant among the grasses, and the ferns among the herbs; (2) In the small gaps, the dominant

Table 4-2. Significant variables in the analysis of herbs, grasses and shrubs in forest gaps of different sizes.

Variable	Forest	Gaps		p-gap	p-dir	p-int
		Small	Large			
Grass cover (%)	24.2	34.0	38.3	0.0391	0.8228	0.7086
Herb height (cm)	53.8	65.2	75.0	0.0001	0.2173	0.0722
Shrubs plus grass cover (%)	39.6	46.3	52.4	0.0488	0.9360	0.4087
Grasses plus herbs cover (%)	37.9	47.1	54.1	0.0029	0.6644	0.9931
Total cover (%)	53.3	59.4	68.3	0.0020	0.6957	0.9088

p-gap, p-dir, and p-int are the p-values (from proc mixed) for gap type, cardinal direction and their interaction.

groups were oaks, *Andropogon*/wire grass, and *Asteraceae*; (3) In the large gaps, oaks, *Andropogon* and *Asteraceae* were dominant.

Predicting Survival

Survival (dependent variable) was negatively correlated with GLI (independent variable) for bare root seedlings (r^2= -0.32999, p=0.0100), but linear regression models were not significant (e.g., p=0.2511, r^2=0.1327, adjusted r^2=0.0346). Containerized seedlings exhibited stronger and also negative correlations between GLI and survival (r^2= -0.7209, p=0.0001), with the following multiple regression model (p=0.0001, r^2=0.5829, adjusted r^2=0.5258):

$$S = 80.983 - 0.862(GLI) + 0.031(WC) - 0.221(GC) + 0.062(HC) + \\ + 0.005(GLIGC) + 0.012(GLIHC) - 0.033\ (GCHC),$$

where: S= survival(%), GLI=gap light index, WC=shrubs cover (%), GC=grass cover (%), HC=herbs cover (%), GLIGC=interaction among GLI and GC, GLIHC=interaction among GLI and HC, and GCGH=interaction among GC and GH.

Biomass

Biomass comparisons were made for dry weight at planting time compared to dry weight one year later for the three nursery N treatments. In bare-root seedlings, stem, tap root and root dry weights all increased with time. Only stem weights had differences due to N treatments (Table 4-3). However, mortality in parts of seedlings (e.g., part of the needles) resulted in a reduction in dry weight one year after planting. Shoot dry weight increased with increasing N levels. However, the total seedling biomass exhibited no difference between planting time and one year later (Table 4-3).

For the containerized seedlings, stem, lateral roots, tap root, root and total dry weights were different between the initial planting time and one year later (Table 4-3). Fertilization at either level increased needle dry weight significantly, while the low N level resulted in the highest tap root. Shoot weights were independent of time and N treatment (Table 4-3).

Table 4-3. Dry weights (g) at planting and one year later by seedling type and fertilization treatment.

Variable	Time		p-time	Fertilization treatment			p-fert	p-int
	0	1		0	2	4		
Bare-root seedlings								
Needle	8.8	5.1	**0.0004**	6.1	7.0	7.8	**0.0528**	0.5907
Stem	1.8	2.5	**0.0001**	1.8	2.2	2.3	**0.0001**	0.2741
Shoot	10.6	7.6	**0.0017**	7.9	9.3	10.1	**0.0300**	0.4692
Lateral roots	3.2	3.3	0.4986	3.0	3.3	3.3	0.5470	0.1628
Tap root	4.8	6.2	**0.0016**	5.2	5.5	5.8	0.1919	0.5235
Roots	8.0	9.5	**0.0010**	8.2	8.9	9.1	0.3229	0.3779
Total	18.6	17.1	0.1252	11.1	18.2	19.2	0.0806	0.3898
Containerized seedlings								
Needle	2.7	2.6	0.4300	2.3	2.8	2.7	**0.0177**	0.7919
Stem	0.5	1.0	**0.0001**	0.7	0.8	0.7	0.7592	0.8154
Shoot	3.2	3.5	0.0706	3.1	3.6	3.4	0.0744	0.7896
Lateral roots	1.2	1.4	**0.0162**	1.3	1.3	1.3	0.8089	0.3516
Tap root	1.3	1.9	**0.0005**	1.6	1.8	1.5	**0.0516**	0.6137
Roots	2.5	3.3	**0.0023**	2.8	3.1	2.8	0.0956	0.3191
Total	5.7	6.8	**0.0032**	5.9	6.6	6.2	0.0701	0.6088

p-time is the p-value for times 0 and year 1, p-fert is the p-value for N fertilization treatments in the nursery during the fall, and p-int is the p-value for the time by N treatment interaction.

Relative Growth Rate (RGR)

In bare-root seedlings, most of the RGR variables were independent of location or N treatment (Table 4-4). The negative RGR values are due to loss of biomass from mortality.

Table 4-4. Relative growth rates (g/g/yr) for bare root and containerized seedlings.

	Nursery N applications				Gap				
Part	0	2	4	p-value	Forest	Small	Large	p-gap	p-NG
Bare-root seedlings									
Needle	-0.73	-0.52	-0.56	0.1039	-0.67	-0.71	-0.43	0.1220	0.3424
Stem	0.27	0.39	0.26	0.4042	0.21	0.27	0.43	0.2440	0.5534
Shoot	-0.48	-0.29	-0.36	0.1722	-0.45	-0.46	-0.23	0.1556	0.3834
Tap root	0.20	0.29	0.23	0.6005	0.21	0.18	0.34	0.1928	0.3618
Lateral root	-0.04	0.17	-0.16	0.1767	-0.13	-0.10	0.20	0.1060	0.2955
Root	0.12	0.24	0.10	0.4335	0.09	0.08	0.29	0.1283	0.2901
Total	-0.17	-0.03	-0.14	0.3472	-0.18	-0.19	0.03	0.1380	0.3392
Containerized seedlings									
Needle	-0.04	-0.10	-0.07	0.1245	-0.16	-0.08	0.04	0.8521	0.1784
Stem	0.71	0.65	0.62	0.6667	0.55	0.69	0.74	0.0894	**0.0345**
Shoot	0.13	0.06	0.07	0.6848	-0.004	0.08	0.18	0.1075	0.1206
Tap root	0.30	0.28	0.43	0.5290	0.25	0.33	0.45	0.0379	**0.0242**
Lateral root	0.21	0.07	0.23	0.3958	0.15	0.16	0.20	0.8582	0.8147
Root	0.26	0.19	0.34	0.2457	0.20	0.25	0.34	0.1902	0.1965
Total	0.19	0.12	0.19	0.5286	0.09	0.17	0.25	0.0998	0.1702

p-value is the p-value for fertilization treatment, p-gap is the p-value for gap types, and p-NG is the p-value for the interaction among N treatments and gap types.

Large Gaps

Survival

In the individual analysis by seedling type, the survival of bare-root seedlings was independent of distance from the center of the gap, with average survivals of only 6.0%, 7.9%, and 12.4% for center, middle, and edge areas of the gap, respectively (p=0.2388). There was also no effect on survival for cardinal direction across the gap, N treatment, or any of the interactions.

The containerized seedlings had higher survival than bare root seedlings, and survival was influenced by the cardinal direction by distance interaction, with highest survival at the southern edges of the gaps (p=0.0020) (Figure 4-6).

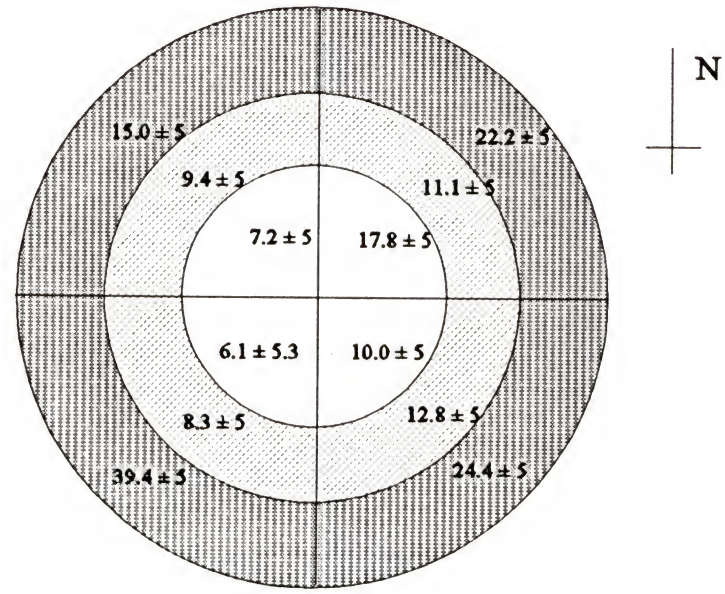


Figure 4-6. Average survival (%, ± standard error) of containerized seedlings in large gaps (144 m diameter) after one year of planting by cardinal direction and distance.

Seedlings planted at the edge of gaps had the highest survival, particularly in the SW cardinal direction (Figure 4-6). In all directions (except in the NE), seedlings planted in the center had the lowest survival. The Pearson's correlation coefficient among survival and distance was -0.72 (p=0.0001).

In the pooled analysis across seedling type, survival was affected only for the interaction between cardinal direction and distance (p=0.0132), with similar trends for containerized seedlings. The Pearson's correlation coefficient for survival and distance was -0.53 (p=0.0001).

Morphological variables

In the case of bare-root seedlings, the bud diameter was dependent on the cardinal direction and the distance by N treatment interaction (p=0.0413). For NE and SE plantings, at all distances, high N treatments had the largest bud diameters. For NW plantings, in the middle area of the gap, the largest values were at the low N fertilization, while the largest sizes were in the no N treatment at the edge of the gaps. For the SW direction, in the center of the gaps, the bud diameters were similar among N treatments, in the middle low N had the smallest values, and at the edge, low and high N levels resulted in the largest bud diameters.

Relative tap-root dry weight was affected by the interaction of cardinal direction and distance (p=0.0255). At the NE, the middle and edge seedlings had the highest root weights (both with 0.41), and the center had the lowest (0.38). In the NW, the middle of the gaps had larger values for tap root weights than the edge (0.43 and 0.31). The SE center seedlings had the greatest relative tap root dry weight (0.46, when compared with

edge and middle seedlings with 0.39 and 0.38). The SW middle seedlings were the largest (0.42), in comparison with the center and edge (0.31 and 0.34).

For containerized seedlings, bud diameter was influenced by the interaction of three factors: cardinal direction, distance, and N treatment ($p=0.0481$). In the NE at all the distances, high N treatments had the largest bud diameters. In the NW at the center, N increased bud diameters. However, at the NW-edge the highest value was found for low N and in the NW-middle plantings, the control had the largest buds. In the SE-central planting, high N treatments had the higher bud calipers, but at the SE-middle plantings no N and high N exhibited the largest buds. At the edge in the SE, the maximum was found at the level no N. To the SW at the center, high N had the largest bud diameter, while in the SW-middle plantings that variable increased with N treatment, and at the SW-edge planting, the no N level was slightly higher than N applications.

Root length was influenced by the distance by N treatment interaction ($p=0.0408$). In the center of the gaps, level 0 of fertilization had a root length of 22.0 cm, while levels high N and low N had 24.8 cm and 20.6 cm. In the middle of the gaps, the no N treatment had the largest root length (25.1 cm), followed by low N (21.7 cm) and high N (18.5 cm). At the edge, high N was superior (27.8 cm) over low N (23.3 cm) and no N (22.6 cm).

Shoot relative weight was affected by the cardinal direction by distance by fertilization treatment interaction ($p=0.0241$). In the different planting areas, diverse trends were found, e.g. in the NW-center and NW-middle, the highest shoot relative

Table 4-5. Effects of the interaction among distance, direction and nursery fertilization treatment on the relative shoot weight (RSW) of containerized seedlings one year after planting (p=0.0241).

Dir	Dist	Fertil	RSW	Dir	Dist	Fertil	SRW
NE	ce	0	0.52	SE	ce	0	0.46
NE	ce	2	0.60	SE	ce	2	0.53
NE	ce	4	0.56	SE	ce	4	0.46
NE	ed	0	0.54	SE	ed	0	0.54
NE	ed	2	0.50	SE	ed	2	0.48
NE	ed	4	0.48	SE	ed	4	0.52
NE	mi	0	0.49	SE	mi	0	0.50
NE	mi	2	0.50	SE	mi	2	0.49
NE	mi	4	0.40	SE	mi	4	0.55
NW	ce	0	0.53	SW	ce	0	0.48
NW	ce	2	0.47	SW	ce	2	0.44
NW	ce	4	0.42	SW	ce	4	0.50
NW	ed	0	0.50	SW	ed	0	0.51
NW	ed	2	0.60	SW	ed	2	0.54
NW	ed	4	0.42	SW	ed	4	0.52
NW	mi	0	0.58	SW	mi	0	0.39
NW	mi	2	0.45	SW	mi	2	0.52
NW	mi	4	0.51	SW	mi	4	0.44

Dir=direction, NE=Northeast, NW=Northwest, SE=Southeast, SW=Southwest, Dist=distance, ce=center, ed=edge=mi=middle sector, Fertil=Fall nursery N fertilization treatment, 0=no application, 2=2 applications, 4=4 applications. SRW=shoot relative weight.

weight was found when no N was applied, while in the NW-edge and SW-middle, the maximum was found at low N. Moreover, in the SE-middle, the highest shoot relative weight corresponded to high N (Table 4-5).

Bud break and herbivory

Bud break timing and needles consumed by animals for each seedling type did not vary with distance, direction nor N treatment (Chi-square test), but in the pooled analysis,

the proportion of seedlings with partially consumed needles decreased from the center to edge of the gaps, with 30.5%, 21.4%, and 16.9% loss, respectively (p=0.046).

Interactions with understory plants

Grass density was influenced by the cardinal direction by distance interaction (p=0.0442). In the NE, the highest grass density was in the middle of the gap (9.3/m²), followed by the center (7.8/m²) and edge (7/m²). In the NW, density increased from the center to edge, with 7.4/m², 8.7/m² and 10.6/m². In the SE the density values from center to edge were 8.3/m², 7.8/m², and 8.8/m². In the SW those values were 11.2/m², 7.7/m², and 8.3/m², respectively.

Total plant cover (grasses + herbs + shrubs) was also affected by the interaction of cardinal direction with distance (p=0.0226). In northern quadrants, the edges had the lowest total vegetation cover (NE: 65.8%, 70.8%, 60% for center, middle, and edge; NW: 71.7%, 70.8%, 61.7% for center, middle and edge). In all the directions, except for the SE, the central or the middle areas of the gaps had the highest total plant cover (SE: 69.2%, 65.8%, 74.2%; SW: 80.8%, 63.3%, 65.0%). In the non parametric analysis, the only association found was herbs increasing with distance from the center of the gap (p=0.042, Chi-square test). Ferns and *Fabaceae* increased from the center to the edge. *Asteraceae* declined from the center to edge. *Convolvulaceae* only appeared as dominant at gap edges.

Bare-root and containerized seedling survival was negatively correlated with grass cover (r = -0.23, p=0.0098), grass height (r = -0.30, p=0.0009), grass density (r = -0.20, p=0.0275), and grass covering plus herbs (r = -0.22, p = 0.0121). These relationships

suggest competition between the seedlings and other understory species. In the separate analysis by seedling type, bare root seedling survival was negatively correlated with grass cover ($r = -0.25$, $p = 0.0497$), total plant cover ($r = -0.28$, $p = 0.0284$), shrub cover plus grass cover ($r = -0.27$, $p = 0.0371$), and GLI ($r = -0.33$, $p=0.0100$). For containerized seedlings the correlations (all negative) with survival were with grass cover ($r = -0.34$, $p = 0.0071$), grass height ($r = -0.45$, $p = 0.0003$), total plant cover ($r = -0.25$, $p = 0.0576$), grass cover plus herbs cover ($r = -0.34$, $p = 0.0075$), and GLI ($r = -0.72$, $p = 0.0001$).

Discussion

Survival of Seedlings

Survival was higher for containerized seedlings than for bare-root seedlings, as has been shown in other studies (e.g., Barber and Smith 1996, Barnett et al. 1996). However, survival was higher under the forest canopy than in small or large gaps, contradicting other studies. For instance, natural longleaf pines had higher survival in areas of low adult density (Wahlenberg 1946, Grace and Platt 1995, Boyer 1993, Platt et al. 1988, Moser 1997), because of its shade-intolerance. The possible reason for these contradictory results is the 1998 climate. The present research was conducted in a very dry and hot year Precipitation in the spring for the period April to June was the lowest in 104 years in Florida (and southern Georgia). During the period, many localities across Florida (and Southern Georgia) received less than half of normal rainfall (generally under 150 mm) (NOAA 2000). Also 110 days of maximum daily temperatures averaging 36°C were observed (International Paper Co., unpublished), so the first sites that in normal years

adversely affect survival, exhibited better growing conditions during this year. An analysis of the main environmental factors may help explain this finding.

Light

The GLI decreased from large gaps to the forest (84.2%, 67.3%, 48.2%), and from the center to the edge of gaps. Light availability in the southern portions of gaps was lower than in the northern parts (McGuire in press), and was inversely correlated to survival. Despite the reported intolerance of longleaf pine to shade, photosynthetically-useful light also reaches shady areas. The forest floor at gap edges receives both diffuse and transmitted light, while the forest (non gaps) receives mainly transmitted light, and the gap center has mainly direct light. Diffuse radiation, relatively abundant at the edge of a gap, is relatively rich in blue wave lengths, the most effective in photosynthesis (Smith et al. 1997). So, even if shade does not represent the highest light condition for longleaf pine seedlings, growth may still occur there.

Temperature and humidity

The greatest extremes in temperature in gaps occurs when gap diameter is roughly equal to 1.5 times the height of surrounding trees, because of the combined effect of shade and ventilation (Geiger et al. 1995). In the central portion of large gaps, with diameters three times the height of surrounding trees, the environmental conditions are approximately the same as those that would be present in much larger openings (Minckler and Woerheide 1965). In our study, the large gap diameter was six times the height of the surrounding trees, so the conditions in the center were similar to much larger gaps.

In these large gaps, survival increased from the center to edge, with the highest value (40%) in the SW-edge sector. In the northern hemisphere, in general, the highest surface temperatures and lowest fuel moisture are on SW slopes of mountains (Schroeder and Buck 1970). Similarly, Randall and Johnson (1998) found that *Pseudotsuga menziesii* (Mirb.) Franco, *Abies procera* Rehd and *Pinus flexilis* James, that S and SW aspects had reduced first and/or third year survival. Schneider et al. (1998) showed that the NE-facing slopes were generally more cool and moist and more favorable for seedlings of *Pseudotsuga menziesii* (Mirb.) Franco and *Pinus ponderosa* Dougl. Ex Laws. survival. A gap is the inverse of a small mountain. In a gap, the absence of the SW slope makes the radiation generally reside for more time in the year in the NE sector giving the SW sector more humidity. Increases in temperature lead to lower relative humidity, and higher amounts of incident light are associated with higher temperatures (Figure 4-7).

Interactions with understory plants

In this experiment, there was a dual effect of other plants and trees on longleaf pine seedling survival. Large trees could have a negative or a positive effect on the seedlings, depending on the conditions. The term "competition" in the present study is defined as the direct interaction of two plants attempting to exploit the same limiting resources (Tilman 1987, Connell 1990). In normal years, fine root competition from adult trees affects young seedling survival, but in droughty years shade may reduce evapotranspiration and improve survival. In a circular gap, factors controlled by competition with adult trees at the edge, such as diffuse solar radiation, air movements and outgoing radiation, should operate in a concentric pattern. Factors related to the slanting

rays of direct solar radiation will follow crescentic pattern (Smith et al. 1997) (Figure 4-8). The competitive effects on nutrients and water created by the fine roots of adult trees extend as much as 16 m into gaps (Boyer 1963, Brockway and Outcalt 1998). Natural longleaf pine regeneration is clustered near the center of gaps, encircled by a wide zone where they are excluded due to the fine root competition and shade (Brockway and Outcalt 1998).

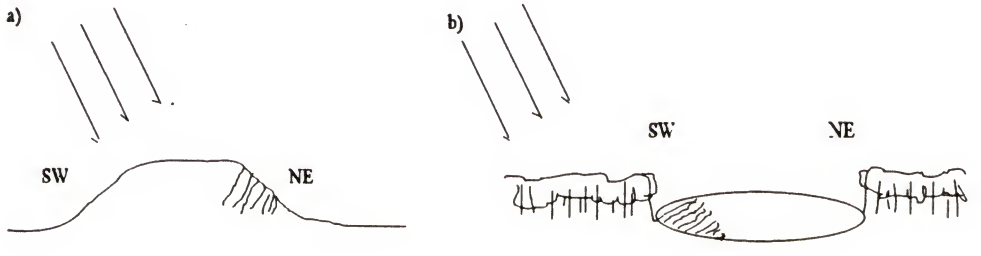


Figure 4-7. In the Northern hemisphere, during the year, the area with lowest temperatures and highest humidity (shade) on a mountain is the NE (a). In a flat large gap the area at the SW edge (because of the trajectory of the sun along the year and the shade of tree wall) will have the lowest temperatures and highest humidity (b). The arrows represent solar radiation along the year.

In our study with droughty conditions, grasses had a negative effect (competition), and shrubs had a positive effect (nurse effect). Shade protects longleaf pine seedlings from high soil temperatures and desiccating conditions (Myers 1992). Grass alone is less effective than scrub oak in lowering soil temperatures. The shade from oaks, when moderate, favors seedling establishment by retarding loss of soil moisture and preventing soil overheating (Wahlenberg 1946). In the multiple linear regression model from our research to estimate survival in containerized seedlings, effects of GLI and grass cover on survival were negative.

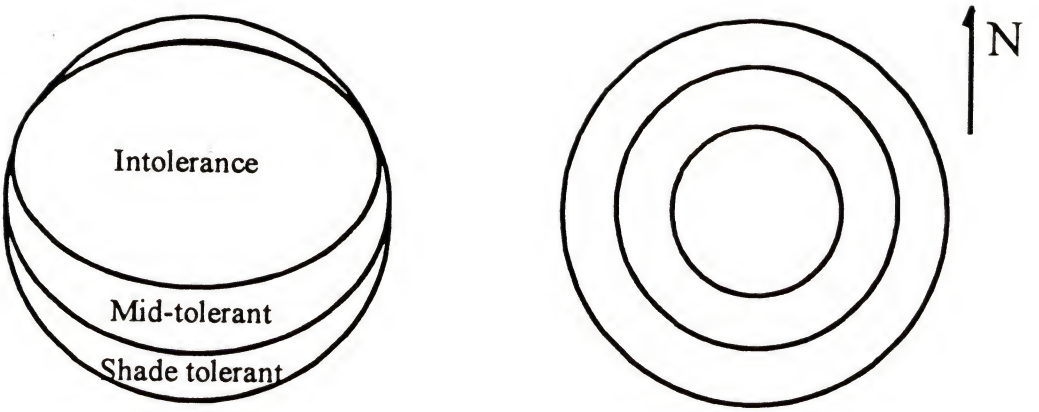


Figure 4-8. Crescentic (left) and concentric (right) distribution of growing resources in gaps in the northern hemisphere (adapted from Smith et al. 1997).

In this study, it was hypothesized that GLI would be positively related with survival, but the drought affected this and the correlation was negative and highly significant. In other words, during very dry years, GLI may provide an explanation for "unusual" survival.

Many grasses have morphological and physiological characteristics, such as a fibrous root system and C4 photosynthesis, which make them tough competitors. Some overtop pine seedlings, reducing the availability of light (Minogue et al. 1991, Morris et al. 1993). Grasses deplete moisture from the rooting zone of young conifers (Cleary et al. 1982). For instance, *Pinus radiata* D. Don doubled its uptake of N after the removal of pasture competition.

Shrubs tend to deplete moisture also, but usually from deeper in the soil profile (Cleary et al. 1982). In our model, survival of containerized seedlings was positively

related to shrub vegetation. There are several examples in the literature of nurse trees aiding survival. Wahlenberg (1946) and Cleary et al. (1982) cite that temperature extremes are moderated by brush, with air and soil temperatures under brush 11 to 17°C lower than in adjacent openings. Hydraulic lift of deep soil water to the surface may also be active in this system, as has been seen for other droughty soils. *Artemisia tridentata* roots cause shallow water depletion during the day, but by night the liquid absorbed from soil by deeper roots is transported to and lost from roots in upper and drier soil layers (Richards and Caldwell 1987, Caldwell and Richards 1989, Caldwell et al. 1998). If this type of water movement was caused by shrubs in our experiment, water taken up by shrubs and even adult trees may have become available to the longleaf pine seedlings.

It is important to recognize that site differences and weather variation, along with species differences in above-ground and below-ground allocation, will alter the mode and intensity of competition at a site from year to year (Burton 1993). For instance, seedlings of *Pinus strobus* L. had N as the most important resource limiting growth one year, but the next year light was the most important limiting factor (Elliott and Vose 1995). Similarly, the survival patterns observed among gaps and within a large gap will vary because the limiting factors (e.g., temperature and water availability) will change from year to year.

In another ongoing experiment in the same gaps, second year survival of containerized longleaf pine seedlings dropped from more than 50% to less than 10% during the 1998 drought (McGuire et al., in press). In that experiment, herbicide was

applied. So, in our case, perhaps the shade of vegetation may have helped to lessen the extreme temperatures.

Soil

A relatively poor soil helps explain the possible influence N fertilization had on some traits one year after fertilization. Pine flatwoods and dry prairies in the southeastern USA have low organic matter contents (except in infrequently burned areas) and low weatherable minerals (e.g., Gholz and Fisher 1982). In the same gaps as our experiment, McGuire et al. (in press) found that immediately after the 1998 drought, extractable N (NO3-N + NH4-N) was higher in gaps (1.52 kg/ha small gaps and 1.53 kg/ha for large gaps) than under the forest canopy (1.16 kg/ha). Net nitrification increased from gap edges to the centers, and N availability increased exponentially with decreasing overstory basal area (McGuire et al. in press). The hardpan layer (present at many locations at 30 cm), may retard water drainage, may be is a physical barrier for root penetration, and may bind nutrients that leach and become unavailable for a long time as part of the hardpan (Snedaker and Lugo 1982, Abrahamson and Harnett 1992).

Fire

In the Brockway and Outcalt (1998) study, regeneration of natural seedlings was excluded from a 4 m wide band along the edge of gaps. There are higher litter loads present there (two times more than in the center of gap), making fires in this part of the gap more intense (Platt et al. 1988, Grace and Platt 1995, Farrar 1996, Brockway and Outcalt 1998), and reducing natural seedling survival.

Morphological Variables and Biomass

In the present study, bud break in the spring was earlier at high N for bare-root seedlings as it was for *Pseudotsuga menziesii* (Mirb.) Franco (Margolis and Waring 1986). Also, bud break in containerized seedlings occurred first at large gaps, then in small gaps, and then in the forest. Similarly, Pelt and Franklin (1999) found (for *Pseudotsuga menziesii* (Mirb.) Franco and *Tsuga heterophylla* (Raf.) Sarg. seedlings) a concentric pattern of bud break occurring earliest towards gap centers and latest at the edges, with increasing direct light and length of the growing season at the center.

In large gaps, root length of containerized seedlings tended to be shorter at higher N under high temperatures (middle area), but seedlings with the high N fertilization treatment had the longest root length at the edge under lower temperatures. Under water stress, nutrient stress, or increased temperatures, cell expansion is more adversely affected than photosynthesis, thus producing excess carbohydrates that trees store (Landsberg and Gower 1997). The proportion of carbohydrates allocated to root growth is higher when water and nutrients are limiting (Commeau and Kimmins 1989, Landsberg and Gower 1997).

In bare-root seedlings, N fertilization in the nursery continued to result in higher shoot biomass and lower relative tap root weight in the field. In containerized seedlings, the shoot:root ratio was lower at high N treatments in small gaps, consistent with previous studies (Waring and Running 1998). Growth rates are correlated to N status under an appropriate nutritional balance (Ågren and Ingestad 1987, Waring 1989). A decrease in N supply will result in a decrease in N in roots and shoots. Yet reduced N availability

produces a relative increase in C allocation to roots (Landsberg and Gower 1997). Lambers et al. (1998) mention cytokinins as the cause of this behavior. In their explanation, in high N environments there is a high rate of cytokinin synthesis in roots, which is exported to the leaves. As a result, photosynthetic capacity and leaf expansion rate increases, requiring a high rate of sugar consumption. The low rate of sugar export results in a slow root growth rate. Conversely, under low N conditions, the same relationship results in rapid root growth rates.

Growth

Relative growth rates of seedlings outplanted in Georgia were very low compared to the seedlings grown from the same seed source under exactly the same nursery culture and container type, but planted in Florida with an application of herbicide (see chapter 3). Palik et al. (1997) note that growth of longleaf pine seedlings increased significantly in the center of gaps. Boyer (1963) mentions that growth of seedlings declines as overstory density increases. In the present research, in the large gaps during this droughty year biomass loss of bare-root seedlings in the center and miccle of the gaps was observed. In the case of containerized seedlings, there was higher growth at the edge, most likely due to greater shading and protection from excessive temperatures. In containerized seedlings, buds broke first in the forest, then in small gaps, and lastly in large gaps, following a gradient of less harsh to more harsh conditions. Khan et al. (1996) found similar results in *Pseudotsuga menziesii*, where both very low and very high levels of humidity delayed bud development.

Management Implications

Palik et al. (1997) proposed the use of circular gaps larger than 0.14 ha to maximize longleaf pine seedling growth, because N and light availability are maximized. Putting together these results with the results of our research, where the highest survival was at the SW edges of large circular gaps, we propose another approach to maximize both survival and growth, using oval-shaped gaps. The longitudinal axis of the oval would

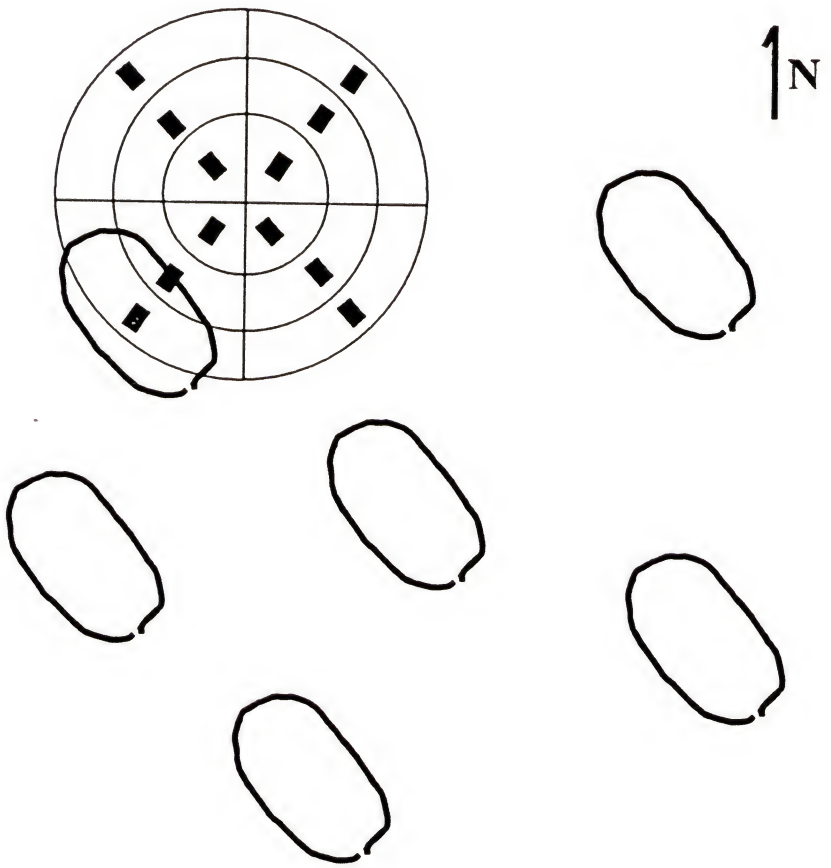


Figure 4-9. Oval-shaped patches larger or equal than 0.25 ha, oriented from NW to SE in its longitudinal axis may maximize the survival and growth of longleaf pine regeneration. The idea is to match the oval-shaped 0.25 ha gaps with the SW edge of the 1.6 ha circular gaps, where the survival was higher.

be oriented from NW to SE, in such a way that includes most of the SW edge part of a large gap (Figure 4-9). Increasing the width of the oval will decrease the shade of the tree wall, increasing survival and growth in non-droughty years. The size of these oval gaps ranges from 0.25 to 0.50 ha, matching the minimum size proposed by Palik et al. (1997).

Conclusions and Recommendations

According to the literature, longleaf pine seedlings survive better in the central area of gaps, beyond a 16 m wide belt at the edge where fine root competition from adult trees, shade from the overstory and more intensity in surface fires exclude regeneration. However, in this research using artificial regeneration, we found that during an extremely dry year (1998), the shadier area near the edge of the gap was the best area for seedling survival. During extremely dry conditions, the center of gap limits the survival of seedlings. In normal years, seedlings survive better in gaps than under canopies (Boyer 1963, Brockway and Outcalt 1998). However, in the especially dry year for this study the opposite occurred.

Several shoot and root morphological traits were shown to be improved by fall N nursery fertilization, even one year after planting. Shoot:root ratio were higher at higher levels of fertilization where the drought was less severe (forest and small gaps, but not in large gaps). Also, bud break was earliest at the sites with less harsh conditions (forest), followed by those with harsher conditions. Apparently part of the understory plants (grasses) affected negatively the survival of seedlings because of competition, while shrubs provided a protective environment against the extreme temperatures, increasing survival.

The availability of the many environmental resources in gap regeneration operate not only in a spatial scale, but also in a time scale, and their effect may change radically when the most crucial environmental variables (e.g., water availability) changes. When this happens, the sites that are normally harsh may become the best for seedling establishment during extreme drought events. During those extreme years in large gaps the approximately 16 m wide exclusion zone could be re-named the "survival zone" (excluding the approximately 4 m wide highest litter-fuel accumulation zone), particularly in the SW portion. Oval-shaped gaps with a surface area larger or equal to 0.25 ha and oriented from NW to SE, may maximize both survival and growth of longleaf pine seedlings.

Because it improves performance-related traits at planting sites under difficult conditions (e.g., drought, competition), N nursery fertilization is recommended. Among those traits there may be faster bud break that may represent an advantage in a competitive environment. Also, a larger shoot dry weight may provide more resistance against physical agents, plus plant biomass has been related with good field performance. Some of this improved traits may mean that N-fertilized longleaf will have a shorter grass stage. However N did not increase stem diameter in our study, and longleaf pine starts to leave the grass stage after reaching 2.5 cm in that variable (Boyer 1990).

We recommend further research with N fertilization. In addition, comparing or modeling gap sizes, shapes and orientations across forest sites in the south would be very beneficial.

CHAPTER 5
ROOT GROWTH POTENTIAL OF LONGLEAF PINE SEEDLINGS

Introduction

Root growth potential (RGP), the ability of seedlings to initiate and elongate new roots when placed in an environment favorable for root growth, is frequently related to field survival (Ritchie 1985). Among the several morphological and physiological traits for estimating seedling quality and performance, RGP remains a useful tool. The use and interpretation of RGP has increased tremendously, since its origin by Stone (1955) (cited by Omi 1991). In the southeastern United States, RGP tests are extensively utilized. For example, the Weyerhaeuser company, through its seedling testing program, performs RGP tests on several species, including longleaf pine (Tanaka et al. 1997).

The objectives of this research were (1) to investigate if the RGP test is a good tool for estimating the field survival of both bare root and containerized longleaf pine seedlings planted under different conditions and (2) to study the effect of nursery nitrogen (N) fertilization in autumn on the RGP.

Methodology

Nursery

Bare-root seedlings were produced at a Florida nursery with sandy well-drained Hogue series soils. The seed to produce the bare-root and the containerized seedlings

were from the same seed lot (ICHLL93) collected in longleaf pine forests in Southern Georgia by International Paper Company.

The seed was mechanically sown at a density of 100/m² in a bare-root nursery in Florida in autumn 1996. The seedlings were irrigated, fertilized, clipped and root pruned as scheduled for longleaf pine production. The seedlings were mechanically lifted in winter 1998 and stored in bundles at 2°C for six weeks.

Containerized seedlings were produced at a Georgia nursery in polyethylene multi-pot seedling trays, with a volume of 107 cm³, and a density of 571/m². The growing medium was a mix of peat moss, vermiculite and perlite. Seeds were spring-sown and the seedlings fertilized and irrigated as scheduled for longleaf pine production in the nursery. The needles were not clipped.

The experimental design for the bare-root experiment was a randomized complete block factorial with five blocks randomly distributed along five nursery beds. Each block consisted of three experimental plots 5 m length, divided by a 0.6 m wide buffer zone. In each experimental plot, 168 kg of ammonium nitrate were applied every other week for 0 (control), 2, and 4 times (no N, low N and high N) during the fall 1997.

The experimental design for the containers was a randomized complete block design factorial with six blocks. Each block had three experimental plots randomly distributed, corresponding to fall N fertilization treatments (100 ppm of N, as ammonium nitrate solution) applied every other week for 0, 2, and 4 times.

Outplanting Sites

Seedlings were planted in two different locations, one in north-central Florida, and the other in southern Georgia. In Florida, the seedlings were planted in a clearcut at the Austin Cary Memorial Forest, University of Florida, Gainesville. The experiment was established at the beginning of April, 1998. Glyphosate herbicide was applied to control competing vegetation. The experimental design was a randomized complete block design with six blocks, each block with six experimental plots, corresponding to all possible combinations of fertilization and seedling type. Each experimental plot had ten seedlings. The total number of seedlings for this experiment was 360. Survival was recorded one year after planting.

The planting site in Georgia was a longleaf pine forest site owned by the International Paper Company. The trees were planted in three different conditions: large gaps, a circular clearcut area with 72 m radius; small gaps, with the same shape but a radius of 18 m; and under a forest canopy (no gap). A total of 8,200 trees were planted in February 1998, in a split-plot experimental design that included six blocks, three gap types, four directions (NE, SE, SW, NW), two seedling types (bare-root and containerized) and the three nursery N fertilization treatments. The large gaps also were divided into a central section, edge and the section between both of those (see chapter 4). No herbicide was applied in this experiment. Survival was recorded one year after planting.

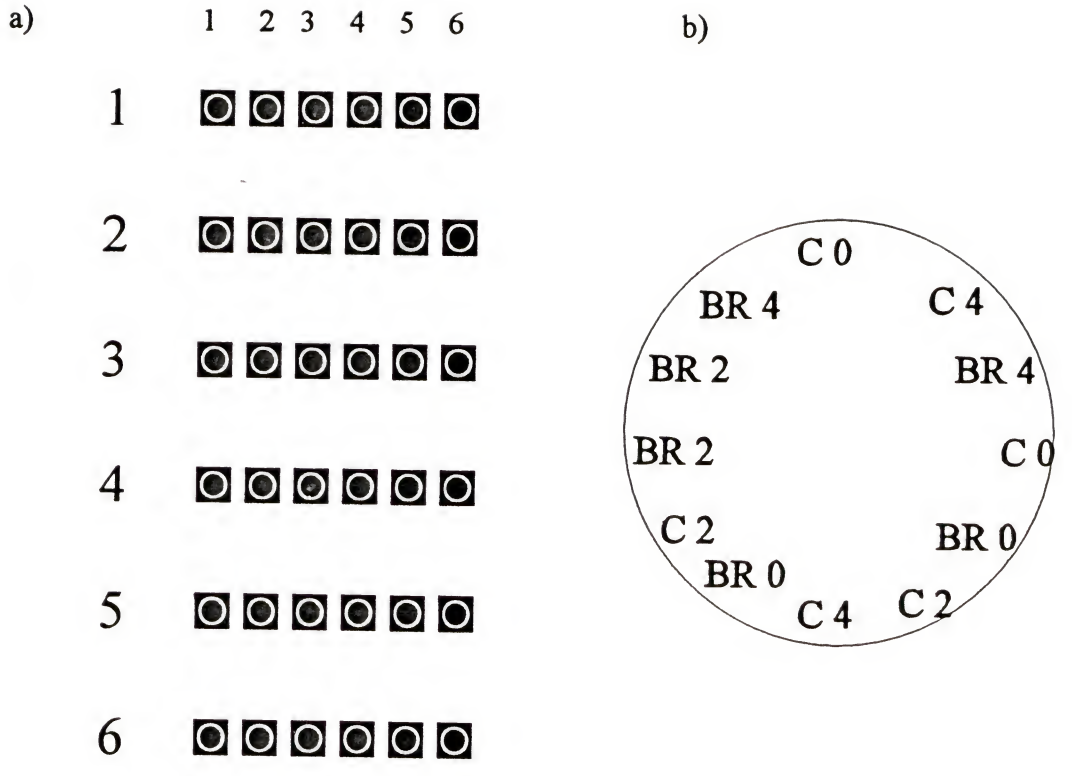


Figure 5-1. a) Six pots (small numbers) per block (large numbers) were arranged in a randomized complete block design. In each pot 12 seedlings were randomly planted. b) Sample of a pot. C=containerized seedling, BR = bare-root seedling, 0= no N, 2 = low N, 4 = high N.

Greenhouse

The RGP part of the experiment was established in a greenhouse at the University of Florida, in April 1998. The experimental design was a randomized complete block design with six blocks. Each block included six 12-liter pots. Twelve seedlings were randomly planted in each pot, including two seedlings of every one of the possible combinations among seedling types and nursery fertilization treatments, for a total of 36 pots and 432 seedlings (Figure 5-1). The growing medium consisted of equal parts of

sand, *Sphagnum* peat moss, and vermiculite. The seedlings were irrigated to field capacity, as needed. The greenhouse conditions were constant light and 28°C.

After one month the seedlings were carefully removed from the pots, by washing off the growing media. The new roots, easily identifiable because of their white color and turgid consistency, were pinched off, placed into paper bags, dried in an oven at 70°C for three days, and then weighed. Seedling caliper and survival were also measured. RGP was expressed as new root dry weight (g).

Statistical Analysis

The data were analyzed with SAS (Statistical Analysis System®), using the mixed procedure (proc mixed), with the degrees of freedom determined by the Satterthwise option (ddfm=satterth) (Littell et al. 1999). Also the fertilization treatment effects on survival were examined using linear and quadratic orthogonal contrasts (coefficients from Montgomery 1997), and the Duncan's multiple ranges test was applied (proc glm). Since the fertilization for containerized and bare root seedlings was not exactly equivalent, the data were analyzed both by seedling type separately and in a pooled data set. Survival was normalized by obtaining the arc sin of the square root of survival divided by 100. Pearson's correlation coefficients were obtained for planting site survival (dependent variable) and RGP in the greenhouse (independent variable), by N treatment and block number, with the correlation procedure (proc corr). In this case, the analysis was performed separately for bare-root and containerized seedlings.

Results

Greenhouse Variables

No differences in RGP were found among N treatments when each seedling type was examined individually. Although there were no stem diameter differences initially, after the RGP experiment the stem diameter of bare-root seedlings was greater under the high N than for the no N treatment (Table 5-1).

In the pooled analysis, RGP did not exhibit differences among seedling types. But despite a p-value larger than 0.05, the RGP showed a quadratic trend, with the minimum value at level 2 of fertilization. The seedling stem diameter was significantly larger at the high fertilization level (Table 5-2).

Overall, survival in the greenhouse experiment was high. The survival in the greenhouse for bare root and containerized seedlings was 98% and 97.7%. However, for the bare-root seedlings the survival was slightly higher at low fertilization. In the case of the containerized seedlings, no differences were found (Table 5-3). In the pooled analysis, neither the fertilization treatment ($p=0.0703$), nor the stock type ($p=0.6965$), nor the interaction between those two factors ($p=0.2819$) were significant.

RGP and Field Performance

The year 1998 was severely dry in the southeastern United States (NOAA 1999). The lack of rainfall and the high temperatures affected the survival of trees planted during the winter 1998. McGuire et al. (in press) and several personal communications report poor survival for longleaf pine planted in that year in the same location or in northern

Table 5-1. Root growth potential and stem diameter at the greenhouse.

Seedling type	Variable	Nitrogen	Mean	p-value	p-lin	p-quad
Bare root	RGP (g)	0	0.43a	0.3090	0.5403	0.1610
		2	0.33a			
		4	0.39a			
	Stem diameter(mm)	0	14.1**b**	**0.0395**	**0.0112**	0.9036
		2	14.6**ab**			
		4	15.1**a**			
Containerized	RGP (g)	0	0.43a	0.3077	0.7334	0.1438
		2	0.31a			
		4	0.40a			
	Stem diameter(mm)	0	11.8a	0.4154	0.5279	0.2490
		2	11.3a			
		4	12.3a			

p-value is the p-value for N treatment, p-lin is the p-value for the linear contrast among N treatments, p-quad is the p-value for the quadratic contrast among N treatments. All of the previous p-values were calculated with proc mixed. For every of the variables into each seedling type, the values with different letter in the same subcolumn were different (p=0.05), according to the Duncan's multiple ranges test (proc glm).

Table 5-2. Pooled analysis for RGP and stem diameter in the greenhouse.

Variable	Nitrogen	Mean	p-nit	Stock	Mean	p-stock	p-int	p-lin	p–quad
RGP (g)	0	0.43**a**	0.0604	BR	0.38a	0.9332	0.9493	0.4921	**0.0236**
	2	0.32**b**		C	0.38a				
	4	0.40**ab**							
Stem diameter (mm)	0	12.9**a**	0.1790	BR	14.6**a**	**0.0006**	0.4760	0.1172	0.3023
	2	12.9**a**		C	11.8**b**				
	4	13.7**b**							

p-nit is the p-value for the comparison among N treatments, p-stock is the p-value for the comparison among seedling types, p-int is the p-value for the interaction stock by N treatment, p-lin is the p-value for the linear contrast among N treatments, p-quad is the p-value for the quadratic contrast among N treatments. All of the previous p-values calculated with proc mixed. For each of the variables, the values with different letter in the subcolumn, are different according to the Duncan's multiple ranges test (proc glm).

Table 5-3. Survival by seedling type and N treatment in the greenhouse experiment.

Seedling type	N treatment	Survival (%)	p-value
Bare root	0	96.7**b**	**0.0199**
	2	99.5**a**	
	4	96.8**b**	
Containerized	0	98.1a	0.6715
	2	98.6a	
	4	97.2a	

p-value calculated with proc mixed. For every type of seedling, the values with different letter indicate differences according to the Duncan's multiple ranges test (proc glm).

Florida. Survival in the Georgia experiment was low (average was 10% for bare root seedlings, 21% for containerized seedlings). However, survival in the Florida planting was better (49% for bare root seedlings, 52% for containerized seedlings). In Georgia, higher survival was obtained in the forest, followed by the small and large gaps (Table 5-4). There was no effect of gap type (p=0.2718), N treatment (p=0.1326), or their interaction (p=0.1220) on the survival of bare root seedlings. For the containerized seedlings, there was an effect of gap type on survival (p=0.0007), but there were no effects of fertilization treatment (p=0.5679) or the gap by N treatment interaction (p=0.9192). In the overall analysis at the Florida planting, there was no difference among seedling types (p=0.6861), among fertilization treatments (52.5%, 55%, 44.2%, for no N, low N and high N, p=0.4147), or for the seedling type by fertilization treatment interaction (p=0.2836). Root growth potential was correlated with field survival in the small gaps at Georgia (Figure 5-2, Table 5-4). However, there were no other significant correlations for survival in the large gaps and forest in Georgia or at the Florida site (Table 5-4).

Table 5-4. Correlations between RGP in the greenhouse and survival one year after planting.

Site	Stock type	Survival(%)	r	p
Edge of clearcut, Florida	br	49.4	0.0776	0.2134
	c	51.7	0.3953	0.7596
Small gap, Georgia	br	9.7	0.2531	0.3109
	c	23.4	**0.5169**	**0.0281**
Large gap, Georgia	br	8.9	0.2132	0.3956
	c	15.4	0.1386	0.5834
Forest, Georgia	br	12.3	-0.1317	0.6025
	c	35.1	-0.1181	0.6408

r = Pearson's correlation coefficient, br=bare-root seedlings, c=containerized seedlings, p=p-value (from proc mixed).

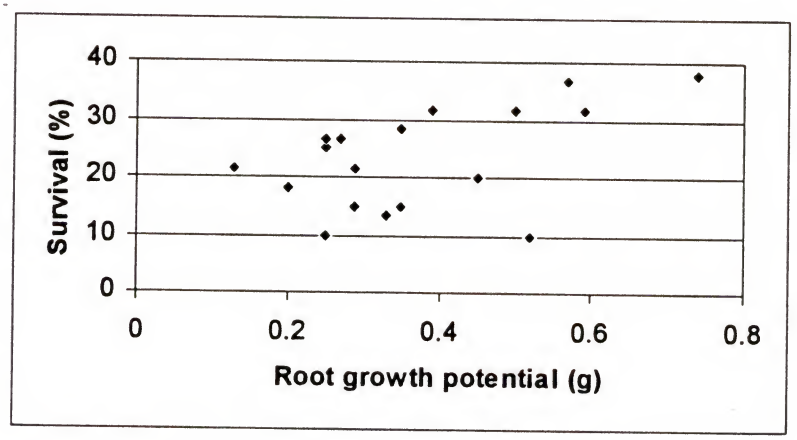


Figure 5-2. Correlation between survival in small gaps and RGP in the greenhouse (r= 0.5169, p=0.0281).

Discussion

Many authors consider RGP a reliable predictor of field survival, but others (e.g. Ritchie and Tanaka 1990) believe that RGP characterizes seedling physiological quality at one point in time, more than predicting field performance. It is assumed that the higher the RGP (e.g. expressed as number of new roots), the better the probability for survival and growth (Johnson and Cline 1991). However, such potential may not be fully expressed when the seedling is planted (Ritchie 1985). For instance, this test seems to be modified by the intensity of shoot dormancy and the strength of the carbon sink in the elongating shoot. When dormancy is weak but shoots are not actively expanding, RGP tends to be high (Ritchie and Tanaka 1990).

Many environmental and plant factors before, during and after the test affect the expression and results of RGP, for example nutrients, soil temperature, mycorrhizae, planting site, choice of root measurement, duration of the test, and use of non-standard conditions (Johnson and Cline 1991, Omi 1991). For example, the root growth potential of longleaf pine seedlings was reduced when Benomyl® was added to Viterra® gel (South and Loewenstein 1994). An interesting point in this regard is that while root growth consumes carbohydrates, new root growth may use reserves or carbohydrates recently produced photosynthetically or old reserves or both? (Duryea and McClain 1984). An approach to this question was presented by Noland et al. (1997), where they found that low light levels reduced photosynthetic rate, root growth potential, and total non structural carbohydrates (mainly starches) concentration in needles and roots of *Pinus*

banksiana Lamb.. This suggests that currently produced carbohydrates are important for RGP expression.

In a paper-debate between Simpson and Ritchie (1997), Simpson points out that, despite methodological inconsistencies in indices, weights, and lengths (Sutton 1990), 75-85% of the literature report direct correlations among RGP and field performance (Ritchie and Tanaka 1990). Ritchie mentions that only when there are positive results in RGP are they published, and that a cause and effect relationship between RGP and field performance has not been demonstrated. Both authors agree that field performance is complex and a function of cultural factors in nurseries, seedling morphology, stress resistance, seedling vitality, and site conditions. Simpson and Ritchie (1997) conclude that RGP's predictive ability increases from mild to harsh site conditions.

Evidently conditions must not be so extreme that they result in almost complete mortality in the field, or impede the manifestation of RGP in the greenhouse. For example, Tinus (1996) found for *Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco, that RGP of seedlings without water stress was greater than the RGP of moderately stressed trees at a range of temperatures, and that RGP of severely stressed trees was zero.

The results of the present research seem to fit the model proposed by Simpson and Ritchie (1997). The containerized seedlings in Georgia had low survival (15%) in large gaps, under the very dry conditions of 1998 and with vegetative competition and the correlation with RGP was not significant. When comparing the small gaps and the forest, the seedlings in the small gaps were exposed to more extreme temperatures. Survival was lower for the seedlings in small gaps (23% vs. 35%) and the correlation between RGP and

field survival was significant. The relatively high survival in Florida (52%) was not correlated to RGP.

The two seedling types had very different root morphology, but there was no difference for RGP among seedling types. According to the results of the present research, despite the original root morphology of each type, the amount (dry weight) of new roots produced will be the same if the two seedling types are grown in the same environment. However, under N-limiting conditions extra supplies of N may increase the root growth. For instance, Prior et al. (1997) studied the development of longleaf pine seedlings under controlled CO_2, irrigation and N conditions. They found that high CO_2 increased biomass only in the high N treatment, and this increase was larger in the roots. Analyzing this and other responses, the authors conclude that the N available in soil is the controlling resource for growth response in the high CO_2 environment. Also, the seedlings with plenty of water grew larger in comparison with water stressed seedlings only in the high N treatment, demonstrating the key role of N.

The differences in results in the present research may also be related to methodology. For example, there may be differences in RGP tests when using operational (seedlings planted in field before obtaining results from RGP tests) and research (seedlings planted after having RGP test data) approaches (South and Hallgren 1997). There may also be different results among species. Survival higher than 70% was associated with RGP levels greater than 10 new roots per seedling in *Picea glauca* Moench (Voss), and *Pinus contorta* Dougl., but not in *Pseudotsuga menziesii* var. *glauca* (Beissn.) (Simpson and Vyse 1995). A RGP greater than five roots per seedling was associated with 80% or

higher survival for *Picea glauca* (Moench) Voss, but survival was unpredictable when fewer roots were produced (Simpson 1994).

Conclusions

Root growth potential of longleaf pine seedlings was not affected by N fall fertilization in nursery, and was correlated with field survival at only one planting site (small gaps) in Georgia. Because of the variable success of using RGP as a predictor of survival, further research is necessary to define the planting site conditions where it could be used for longleaf. That studies should include non multiple years and variable N treatments.

CHAPTER 6
GENERAL CONCLUSIONS

The results of three experiments in this research (chapters 2, 3, and 4) investigating the effects of nitrogen (N) fall fertilization of bare root and containerized longleaf pine seedlings, lead to some conclusions about reforestation practices. Nursery management practices such as N fertilization may have effects on longleaf pine seedlings that extend for at least one year after the planting date.

In the nursery experiment, very few morphological traits responded to N fertilization. However, the timing of bud burst was affected in both seedling types and this may provide an advantage in competitive environments. Starch reserves concentrated more in roots than shoots, and bare-root seedlings had lower starch concentrations at high N levels, showing that in poor N environments, seedlings accumulated more reserves in the roots.

At the Florida planting site, first year survival was not affected by nursery N treatments. Longleaf is a species with an extended grass stage. One of the characteristics of the grass stage is increased root growth, and in this research project, the root length at planting time (despite the pruning of roots), was correlated with no common survival one year after planting in bare-root seedlings. Moreover, root length was also correlated with stem diameter, stem length, and shoot dry weight (all one year after planting). In containerized seedlings, survival one year after planting date was positively correlated with

primary needle length, shoot length and shoot dry weight at planting time. In both seedling types, the largest increase in biomass after one year of the planting date was in the roots. Containerized seedlings had a larger biomass increase than bare root seedlings, but bare root seedlings remained larger after one year of field growth.

The reforestation experiment in gaps at Georgia was conducted under unusually hot, dry conditions. Under the circumstances, survival in the forest was higher than in gaps, and survival in large gaps was higher at the edge of the gap (exclusion zone), where apparently fine root competition from adult trees, shade from the overstory and larger litter-fuel accumulation reduces the survival chances for longleaf pine seedlings during normal years. During droughty years, survival may be positively affected by the shade of adult trees and the nurse effect of shrubs. An area particularly good for survival in large gaps was the southwest edge. No effect of nursery fertilization on survival was found.

The availability of many environmental resources in gaps operates not only at a spatial scale but also on a time scale. When one key resource such as water is limiting, there is a synergistic effect that may make normally adverse sites the best ones. During those droughty years these areas can be called the "survival zone", instead of the exclusion zone (excluding the 4 m wide zone at the very edge where there is the highest litter-fuel accumulation and more intense fires). The use of oval-shaped gaps, oriented from NW to SE is proposed to maximize both survival and growth of longleaf pine seedlings.

Root growth potential was correlated with survival only for containerized seedlings in small gaps. Further research is recommended on this topic. Lastly, because

of its effect on improving traits related to field performance, we need more research on N fertilization and its effects on survival and the length of grass stage.

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BIOGRAPHICAL SKETCH

Dante Arturo Rodríguez Trejo was born in August 1962 in Mexico City. At age 20 he received a bachelor's degree in Forest Engineering from the Forest Sciences Division, University of Chapingo, Chapingo, State of Mexico, MEXICO.

From 1984 to 1989 he worked as a technician, as Chief of the Forest Office, and chief of the Department of Special Conservation Areas for the Mexico City government, on the 35,000 ha of forests surrounding Mexico City. There he worked in reforestation, forest inventory, environmental interpretation, urban forestry, and fire management. From 1989 to 1990 Dante was employed as Chairman of the Texcoco Region for the State of Mexico government, mostly involved in forest nurseries, reforestation, and fire management work.

In 1989 he enrolled as a partial time graduate student at the Graduate College, Montecillos, State of Mexico, MEXICO. In 1990 he left the operative work and enrolled as a full-time master's student and obtained his degree in silviculture and forest management in 1991.

From 1992 to the present he has been a professor and researcher in the Forest Sciences Division, University of Chapingo, Chapingo, State of Mexico, interested in forest seeds, forest nurseries, reforestation, reclamation, urban forestry, and fire management.

He has published several works on those topics (most in Spanish), including a book on forest fires which received second place in Mexico's National Forest Award in 1998.

In 1996 Dante obtained permission from the University of Chapingo to start his Ph.D. studies in restoration ecology at the School of Forest Resources and Conservation, University of Florida, Gainesville.

Awards: In 1984 Dante attended the II International Fire Management Course at Marana, AZ, obtaining second place in the final exam. In 1989 he obtained the "Mexico City" medal for the capacitation provided to attendants to the International Forest Fires Course in Mexico City. He was selected the best teacher twice in the Forest Sciences Division, University of Chapingo. From 1991 to 1994 he was a candidate for National Researcher with the National Researchers System, and he has also been awarded as a professor by the University of Chapingo.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Mary L. Duryea, Chair
Professor of Forest Resources and Conservation

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Timothy L. White
Professor of Forest Resources and Conservation

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Henry L. Gholz
Professor of Forest Resources and Conservation

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Robert J. Mitchell
Scientist, Joseph W. Jones Ecological Research Center

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Doria R. Gordon
Professor of Botany

This dissertation was submitted to the Graduate Faculty of the College of Agricultural and Life Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May 2000

Wayne H. Smith
Director, School of Forest Resources
and Conservation

Winfred M. Phillips
Dean, Graduate School